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THE RÔLE OF FAT IN THE DIET

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The physiology of two classes of lipids, the phospholipids (1), and the sterols (2) has been considered in recent reviews. The term "fats and oils" as used in this survey refers to the simple lipids or esters of fatty acids with glycerol, sometimes designated as "true fats." Most of the commoner fats and oils contain 98 or 99 per cent of glycerides. In regard to the term "ether extract" which has been considered as representing the fatty component of foods, it is well to remember that it is a complex mixture which may actually have a low fatty acid content. The reports of Chibnall and Channon on cabbage-leaf cell cytoplasm (3) and of Horwitt, Cowgill, and Mendel on spinach leaf (4) strikingly emphasize this point. According to their experiences with improved analytical methods, the "ether extract" of these two green leaf foods contains only 27 per cent and 41 per cent of fatty acids respectively.

Prout (5) wrote more than a century ago that "a diet to be complete, must contain more or less of all of the three staminal principles," i.e., oleaginous, saccharine, and albuminous. This is one of the first expressions concerning the appreciation of the necessity for fat in the diet. Our knowledge concerning the quantitative requirement of dietary fat is incomplete as evidenced in the following pronouncements of students of nutrition: "Fat must be a constituent of the normal diet, but the data at present available do not suffice to permit a precise statement of the quantity required" (6), and, "Standards of fat intake proposed by physiologists show wide variation, as does the actual intake of human beings in various parts of the world. The standard fat allowances of Voit, Rubner and others are about half the actual consumption of average men in Western civilization to-day. Fat has a high satiety value; its high caloric content reduces the bulk of food consumed; it is the vehicle of fat-soluble vitamins; infants at the breast receive about half of their calories in the shape of fat; it may yet be shown to possess some intrinsic

quality of value to the organism. For these and other reasons, it seems advisable that standards of fat intake should not be too low" (7).

GENERAL CONSIDERATIONS OF FAT IN THE DIET. The significance of fats in the diet has been ably discussed by Starling (8) who served as chairman of the Royal Society Food (War) Committee. After commenting on the rôle of protein and carbohydrate in the diet, he remarked, "The question as to the requirements of the body for fats and the significance of these substances in ordinary metabolism has, however, been practically neglected, and it has needed the restriction of food supplies throughout Europe, which is the immediate consequence of the war, to bring into prominence the great practical importance of the fat supply."

The low daily fat ration in Germany, as little as 20 grams per person at times, was regarded as one of the most severe deprivations of the population. The total energy intake of the dietary was at one period reduced to a point less than is necessary to maintain the body needs. In the words of von Müller (9), "the real caloric value of the diet was not 1300 calories as officially given, but only 1100 to 960 calories on account of the large proportion of unabsorbable material." Near the end of the war experiments were performed with animals and with human subjects in England to determine whether the fatty acids, which remained after separation of glycerol from the original fats and which accumulated in enormous quantities could be used for human consumption. The outcome of these tests indicated that fatty acids when ingested in daily amounts of about 36 grams per individual, could be readily utilized by humans (10, 11). Lepkovsky, Ouer and Evans (12) who compared growth in rats recently reported that the free fatty acids of lard were good sources of energy when fed as 25 per cent (by weight) of the diet, but somewhat inferior to the glyceride at the 60 per cent level.

Within wide limits, fat and carbohydrate can replace each other in isodynamic proportion. However, as is well known, this property of mutual replacement is not unlimited. The biological importance of dietary fat depends on several factors. It is absorbed more slowly than carbohydrate. A meal lacking in fat is deficient in staying power as was plainly evident during the World War in various civilian populations. Starling (8) recites the following experiences in the year 1918: "There have been complaints from Welsh miners during the first of this year that the food had not enabled them to work their normal shifts. They are used to taking with them to their work pies made of fat pastry with a little meat, and it was impossible to make the pastry in conse-

quence of the absence of fat. Premature hunger was also a universal experience in this country during the first five months of this year." Concerning the factor of bulk of food and the consequences of ingesting large amounts of carbohydrate foods, Starling comments: "The question of bulk is probably one of the most important factors in determining the need for fat. The human alimentary canal has been developed so as to cope with a diet in which 20 to 25 per cent of the energy is presented in the form of fat. In order to get the same energy from carbohydrates the alimentary canal would have to be much larger. Theoretically, then, the absence of fat can be made up by an increased supply of carbohydrates. But this can only be carried out in a certain number of individuals and under certain conditions. The ordinary individual deprived of fat diminishes his total intake of food and exists on a lower metabolic level. It is a notable fact that during the shortage of fat in this country in the early part of 1918, there was no appreciable increase in the consumption of cereals. It was easier to live on the stored-up fat of the body than to adopt a stuffing process with carbohydrates." Furthermore, "it seems that carbohydrates are more subject to fermentative changes in the intestines than fats. Overloading the intestines with carbohydrates in many individuals leads to abnormal fermentation, the production of gases, and general discomfort." McClendon (13), while a visiting professor in Japan, confirmed these latter observations. He writes that he "was much impressed by the low fat content of the Japanese diet and the great amount of fermentative dyspepsia among the Japanese. It seemed to me that men were incapacitated for long periods of time because of the low fat content of the diet."

Evidence has been presented to show that in heavy muscular work there is a greater economy of performance on carbohydrate than on fat. According to a recent report of Christensen, Krogh, and Lindhard (14), subjects on a diet rich in carbohydrate were able to carry on for over four hours whereas on a diet with a high fat content marked fatigue occurred in 90 minutes.

In a standard dietary suitable for the average adult, Atwater proposed that fat represent 33 per cent of the total of 3520 calories, whereas Playfair proposed that in a diet of 3140 calories, the fat component furnish only 15 per cent of energy intake (7). Murlin (15) reports that in the diet of the U. S. army soldier in the training camp, fat represented 31 per cent of total energy actually consumed (about 3700 calories). Data relative to the percentage of total calories supplied by fat in the dietaries of various peoples on the basis of light, moderate or heavy work, have been collected by various investigators (8, 16, 17). "Experience proves

that the percentage of fats in the diet of different people varies greatly and that the influence of climate, locality, habits, work, is very great so that it is impossible to utilize one particular finding for general application" (17). In an interesting discussion of the dietary value of fats Howe (18) comments as follows on the consumption of fat and on the place of fat in the diet: "In the so-called standard dietaries the weight of the fat consumed has varied between 50 and 100 gms. per day. The average daily consumption of fats and oils purchased as such in the United States is roughly 63 gms. per capita. Some dietaries in the South have shown a consumption of almost twice as much fat." "Fats and oils have a unique place in the dietary because of their ability to impart flavor, especially richness of flavor, to change texture, and to add attractiveness . . . (Fats) have the fortunate faculty of increasing our enjoyment of food."

In a stimulating review on the qualitative and quantitative aspects of nutrition, Hamill (19) refers to the observations of Voit and of Cathcart and Murray regarding the relation of income and fat consumption. More than 50 years ago Voit stated that "as a general rule the diet of the well-to-do classes contains both absolutely and relatively more of the dear fat and less of the bulky carbohydrates," and from the recent study of the British workers, Cathcart and Murray, it was found that "there is an increase of fat with rising income which is more marked than the rise in protein."

The necessity for consideration of sufficient fat and carbohydrate to provide for an ample energy intake in the dietaries of children, is emphasized by Rose (20) in an interesting essay on "What is Progress in Nutrition?" She remarks: "Some would seem to rest content with the idea that all the problems of nutrition can be solved by the simple formula, 'eat more milk and vegetables.' Of course no one will deny the importance of increasing both of these in the American dietary but it is not safe to assume that an ideal diet will result. Study of the dietaries of several groups of children, all having an excellent supply of milk, and none being very low in vegetables, has shown that the commonest single dietary defect is shortage of total calories, sometimes through too low a supply of fat and sometimes through a very limited intake of cereal foods. Furthermore, such a simple formula does not protect the appetite and digestion."

The fat supply has been one of the recent major nutritional problems in Germany. In a discussion of the dietaries of German industrial workers v. Tyszka (21) remarks on the needs for a fat rich diet, which

can only be procured when fat is reasonable in price. The influence of cost upon margarine and butter consumption is then cited. v. Tyszka also refers to similar considerations of the fat consumption in Czecko-slovakia. In many countries to-day, the general and pressing problem of nutrition is that of harmonizing economic and public health conditions.

In a detailed inventory statement of Germany's food supply (22), published in late 1914, the urgent necessity of conserving fat is vividly presented, and repeated reference is made to "Rubner's demonstration that 20 grams of fat per head of the population, . . . appears daily in the Berlin sewers (which demonstration), can be understood by anyone who has ever noticed how much fat is thrown away . . . even in the kitchens of the working classes." English (10) and American (23) investigators also have reported a greater wastage in edible fat than of other edible foodstuffs. A widespread saving of fat in this country in 1918 led to a reduction of 30 per cent of fat in garbage (24).

In view of these observations on wastage of edible fat and in view of possible inaccurate analyses of some of our food products, apparently our estimates concerning actual consumption of fat should be revised. Morey (24A) emphasizes consideration of wastage of food in calculating the energy values of diets. Horwitt, Cowgill and Mendel (4), who also assayed the crude fiber, protein and carbohydrate constituents, remark that their analytical methods "have been devised with the idea that they be applied not only to the green leaf but to foods and feeds in general." They add: "It is quite evident that if we are to determine the true caloric content of many of our foods, we shall have to change our methods of analysis."

FATS AS CARRIERS OF VITAMINS. Brief attention is now given to another criterion for measuring the food value of fats based upon the knowledge that some fats contain accessory food factors, designated as vitamins, which are indispensable for the nutritive well-being of the organism. The early experiences reported by McCollum and Davis (25), and by Osborne and Mendel (26, 27) paved the way for a broader understanding of the physiological significance of fatty products like butter fat, egg yolk, and cod liver oil which had for a long time enjoyed a popular, though largely unexplained reputation for unique nutritive potency. Since this pioneer work, a large mass of experimental data has been accumulated indicating the manifold ways in which, for example, vitamin A functions. In this connection brief reference is made to one war-time experience in Denmark. Due to a lack of vitamin

A in the diet of Danish children, particularly during 1917, conditions of xerophthalmia and dystrophy developed. These clinical conditions were improved or cured when butter was rationed by the state at such a low price that all could buy it (28). It is beyond the scope of this review to discuss further the deficiency diseases which result from the absence of the various fat-soluble vitamins in the diet. Each deficiency disease has been adequately described in numerous publications.

Investigation has been made of the potency of the vitamin A and D factors in a large number of oils and fats as well as other foodstuffs. Detailed directions for conducting assays of vitamins A and D in cod liver oil and minimum requirements of vitamin activity (600 U. S. P. units of vitamin A per gram, and 85 U. S. P. units of vitamin D per gram) have been published in the last decennial revision of the United States Pharmacopoeia (U. S. P. XI) (29). As carriers of vitamins A and D cod liver oil and halibut liver oil are commonly used medicinal oils. In recent years claims of exceedingly high vitamin D activity have been made for a number of liver oils of fish including the bluefin tuna, yellowfin tuna and swordfish (2).

If margarines or other butter substitutes are adequately fortified with vitamins A and D, discrimination against their use is unwarranted. According to a recent report, "Brands of margarine—enriched and equal in vitamin value to summer butter are now on the market in several countries" (7).

Wheat germ oil has been found potent in the vitamin E factor.

Recently some of the vitamins, not particularly related in their chemical natures or nutritional functions, have been fairly well characterized from the point of view of chemical constitution. Vitamin A is apparently an unsaturated alcohol possessing the formula $C_{20}H_{30}O$ (30). The presence of a β-ionone ring in combination with four conjugated double bonds is the criterion of vitamin A activity in both vitamin A and its precursor, carotene. With respect to vitamin D, two crystalline products which are apparently identical and which possess intense antirachitic activity, have been prepared by different groups of workers. One of these preparations is called purified calciferol (31) whereas the other one has been referred to as the substance "D_2" (32). Both products have a molecular formula which is isomeric with ergosterol. A multiplicity of substances which possess vitamin D activity is now available. Concerning vitamin E, potent concentrates of this factor have been prepared by Olcott and Mattill (33); and Evans, Emerson and Emerson (34) recently isolated α-tocopherol from wheat germ oil as an allophanate; this alcohol possesses marked vitamin E activity.

ESSENTIAL FATTY ACIDS. The work of Lawes and Gilbert (35) in England on pigs and that of Jordan and Jenter (36, 37) in America with dairy cows has given ample proof of the ability of the body to produce body fat and milk fat from other sources than dietary fat. On the basis of this information it might be considered a matter of indifference whether, after provision of the proper amount of protein, fat is present or absent from the diet. In 1920 Osborne and Mendel (38) conducted an inquiry into the requirement of the healthy mammal for fat in the diet. They observed growth in rats fed diets which were presumably adequate with respect to both the fat-soluble and water-soluble vitamins and concluded that if true fats are essential for nutrition during growth the minimum necessary must be exceedingly small.

In 1928 Evans and Burr (39) observed a definitely subnormal weight and irregular ovulation in rats on diets low in fat. A little later, McAmis, Anderson and Mendel (40) who fed a diet of extremely low fat content, observed comparatively good, though by no means optimal growth. The best growth was exemplified by those animals which received some small inclusion of fat (cod liver oil or peanut oil) in their diet. On the "fat free" dietary regimen, the animals presented an unthrifty appearance and evidence of kidney damage was noted in about 50 per cent of the rats. McAmis and co-workers remarked that it is not conclusively demonstrated whether the apparent beneficial effect of a small amount of fat is due to its content of vitamin A or other vitamins, or to its action as a vehicle for the fat-soluble vitamins, or whether fat *per se* is essential.

Simultaneously Burr and Burr (41) presented for serious consideration a hitherto unsuspected possible rôle of certain specific fatty acids in the animal organism. These investigators (42) who also employed a highly purified "fat free" diet, considered "growth, scaliness of the skin, and condition of the kidneys as measures of the effect of various fats in the diet." They assert that vitamin E is not the controlling factor in the disease which they describe. Saturated fatty acids are ineffective, but linoleic acid, either isolated in pure state, or as found in natural oils, is the outstanding curative or prophylactic agent. The hypothesis is advanced that: "Warm blooded animals in general cannot synthesize appreciable quantities of linoleic acid. The synthesis of other unsaturated acids, including linolenic, is probably equally limited."

Previous studies had left the value of oleic acid uncertain, but in 1932, Burr, Burr and Miller (43) proved this acid to be ineffective in the curing of the fat-deficiency disease. In addition they showed that linolenic

acid and linoleic acids are about equally effective and therefore are interchangeable. Other unsaturated acids such as α-eleostearic, an isomer of linolenic acid, and arachidonic are ineffective. As a result of these studies linoleic acid assumes the dignity of indispensability for living animal organisms, and attains a place similar to that of certain amino acids, minerals, and vitamins in being a necessary constituent of a complete diet. Evans and co-workers (44, 45, 46) who confirmed the work of Burr and Burr found that the presence of linoleic acid in the body fat of rats was dependent on its inclusion in the dietary fat. Furthermore, they claimed that successful gestation and lactation was impossible and also that male sterility invariably occurred on "fat-free" diets; the addition to the diet of essential fatty acids not only prevented but also corrected these conditions.

Numerous other investigators have presented evidence to confirm Burr and Burr's work. Borland and Jackson (47) have made a study of the kidney lesions; Sinclair (48) substantiates the results on growth; Tange (49), Becker (50), and Sahashi (51) emphasize the essential nature of linoleic acid; and Green and Hilditch (52) from a study of data on hog and rat body fats as well as on milk fats, conclude that linoleic acid when present in the glycerides of land animals, is a product of assimilation and not of synthesis by the animal. Spadola and Ellis (53) find that the presence of linoleic acid could not be demonstrated in the adipose fat of rats fed on a low fat diet, whereas its presence in the diet to the extent of only 0.2 per cent was easily detectable. They remark that: "the readiness with which this acid is deposited naturally raises the question as to the minimum amount necessary for well being."

In the early studies of Burr and Burr (42) evidence was presented to show that butter fat was not an effective curative agent of the fat deficiency condition. This observation indicated that linoleic acid was either absent or present in very small amounts in this product of glandular activity. Reports regarding the linoleic acid content of butter fat do not agree. Hilditch and Sleightholme (54) concluded that some butter fats may contain as high as 4.5 per cent of the acid. On the other hand Holland and co-workers (55) and Bosworth and Brown (56) failed to verify its presence, and Eckstein (57), who seriously questioned the high figure of Hilditch and Sleightholme, reported that several butter fats examined by him contained approximately 0.2 per cent to 0.5 per cent of linoleic acid and an average of 0.13 per cent of linolenic acid. Eckstein's study demonstrated that the linoleic acid content of butter fat was related to the amount of this acid ingested by the lactating

animal. According to the most recent report of Hilditch and Thompson (58) butter fat may contain approximately six per cent of linoleic acid when linseed oil is consumed by the cow.

The general thesis that linoleic acid is indispensable has been challenged by a number of students of the problem (59, 60). Gregory and Drummond (60) who have made an investigation of fat metabolism with special reference to nutrition on diets devoid of fat, attribute the abnormal condition to a deficiency of one or more factors of the vitamin B complex rather than to an inability to synthesize linoleic acid. In commenting on various studies related to this problem, Burr and Brown (61) remark that differences in technique may readily account for lack of agreement of results. They emphasize that fat deficiency is characterized by a late failure in growth after an early period of rapid growth. This failure is accompanied by a high percentage of kidney lesions, abnormal gas exchange, and abnormal water consumption.

An abnormal respiratory quotient (considerably above 1) was originally noted by Wesson (62) in rats ingesting a fat deficient diet. This was later confirmed by Wesson and Burr (63) and Burr and Beber (64) on fat-free diets. Work reported by Wesson and Murrell (65) indicates the specific metabolic factor is present in fat (lard), but that it is not linoleic acid.

Studies on the essential fatty acids have brought into question the well known theory of fatty acid desaturation by the liver (59, 66). This topic has been reviewed by Williams and Anderson (67) who remark: "That the body is able to synthesize fatty acids of a greater degree of unsaturation than oleic seems evident. While the liver may not be the only site of such synthesis, it appears to be the most important organ concerned in the elaboration of these fatty acids. Linoleic acid appears to be an essential fatty acid. Whether the body is incapable of the high rate of synthesis demanded by the growing organism for this particular fatty acid or whether it is unable to actually synthesize linoleic acid due to the particular position of the unsaturated linkages in the chain, is not evident. The latter would seem to be the more tenable conclusion."

A recent report by Schoenheimer and Rittenberg (68) presents direct evidence of the ability of the organism to desaturate fatty acids. They fed saturated fatty acids labelled with deuterium to mice and fractionated unsaturated fatty acids containing deuterium from the body fat of these animals. Further work is necessary to identify the specific unsaturated fatty acids formed in this desaturation process.

Since the available evidence favors the essential nature of linoleic acid,

consideration has been given to the occurrence of this fatty acid. Burr and Burr (42) indicate the linoleic acid content of a number of oils and fats used in their curative experiments. According to analyses recorded by Jamieson (69) linoleic acid is present in a number of vegetable oils. In olive oil and peanut oil it constitutes nearly 7 per cent and 25 per cent, respectively, of the total fatty acids, whereas in corn, cottonseed, sesame, linseed and soy bean oils, its content ranges from approximately 35 per cent (in sesame oil) to 50 per cent (in soy bean oil). "Hard" lard contains roughly 1 to 7 per cent, whereas "oily" lard may contain as high as 36 per cent of the fatty acid (70); it is also present in egg fat to the extent of 15 to 19 per cent (71). According to Hilditch (72) the following body fats contain this particular fatty acid in the amounts indicated: hen fat (22 per cent), beef tallow (3 per cent) and mutton tallow (4 per cent). Reference has already been made to the linoleic acid content of butter fat. In view of these remarks regarding the widespread occurrence of this so-called essential fatty acid and also in view of the small amount necessary to alleviate the fat deficiency condition, it does not seem warranted to imply (73) that "the badly needed linoleic glyceride" has a limited distribution and that well-being is dependent upon the consumption of any one oil which contains this acid.

The observations of Burr and Burr (42) that skin changes develop in rats fed diets lacking in certain unsaturated fatty acids suggested to Hansen (74) the possibility that infantile eczema might be partly dependent on this type of dietary deficiency. Hansen (75), therefore, fed the so-called essential fatty acids in the form of linseed oil or corn oil to eczematous children and observed improvement in their condition. Cornbleet and Pace (76) also obtained favorable effects with refined corn oil when administered to adolescent patients for a long period of time—12 to 18 months. All reports of treatment of a capricious condition like allergic eczema with linoleic acid containing oils are not in agreement. For example, Taub and Zakon (77) state that they did not find linseed oil to be an effective therapeutic agent for their eczematous patients.

THE VITAMIN B SPARING ACTION OF FAT. Evans and Lepkovsky (78, 79) in 1928 presented the novel problem of the benefit of fats in the vitamin B (antineuritic) deficient diet. Employing dietary mixtures consisting essentially of casein and sucrose with added salts and vitamins, they found that if fat (lard) was added, the amount of vitamin B required to establish any definite level of growth or frequency of ovulation in young rats was always less than was required when fat was

absent. They termed this phenomenon "the sparing action of fat on the antineuritic vitamin." From their early investigations (80, 81) it was found that fats which are approximately equally well absorbed showed no difference in their vitamin B sparing ability. Furthermore, it was demonstrated that in the presence of the vitamin B factor, natural fat is superior to a number of the glycerides of single fatty acids. It was also concluded that the sparing action of fat on vitamin B could not be ascribed to an interaction in the alimentary tract between the fat and the vitamin (82).

Other workers have reported disparity between their results and those obtained by Evans and Lepkovsky. Kemmerer and Steenbock (83) sought to determine whether dietary fat conserves the vitamin B present in the tissues of adult rats, chickens (adult birds) and of pigs. From this investigation it was concluded that the addition of a high percentage of fat in the diet had no effect on the vitamin B content of the animal tissues of any of the species studied. Gregory and Drummond (60) and Drummond (84) have disputed the idea advanced by the California workers. In all of their experiments the test animals (young rats) declined in body weight in much the same manner as when fed on a vitamin B deficient diet, regardless of the proportions of fat and carbohydrate in the diet. In another study conducted by Sure (85) the vitamin B requirements for lactation in the rat were not influenced by the amount of fat included in the diet.

Concerning confirmation of the work of Evans and Lepkovsky, Guha (86) "observed that while olive oil and palm kernel oil do not give any definite indication of a sparing action on vitamin B_1, lard appears to have a definite effect. The decline in weight of rats completely deficient in vitamin B_1 is also slower when lard is present in the diet." Westenbrink (87) who fed pigeons a diet rich in carbonhydrate and free from fat, reported that these birds developed typical symptoms of polyneuritis in less time than pigeons fed a ration rich in fat and free from carbohydrate. The difference in time, regarded as being statistically significant, was attributed to the presence of fat. Westenbrink (88) offers the possible explanation that in feeding of fat the animal organism uses less vitamin B than in feeding of carbohydrate, and that a large addition of fat hinders in some way the onset of polyneuritis.

In the early investigations of Evans and Lepkovsky it was assumed that a simple relationship existed between vitamin B and the level of fat in the diet. However, in a later inquiry Evans and coworkers (89) indicate that the sparing action of fat on vitamin B is not so simple a

phenomenon as they had formerly supposed and that protein and vitamin G as well as fat participate in sparing vitamin B. In a subsequent study Evans and co-workers (90) observed the rate of growth of rats fed diets which were rich in fat and deficient in the vitamin B factor, and which contained liberal amounts of protein (casein 36 per cent) and vitamin G (autoclaved yeast 10 per cent). By way of summary these investigators remark that "with reference to their ability to spare vitamin B, fats can be arranged as follows in the order of their effectiveness: coconut oil, lard, crisco (hydrogenated cottonseed oil), butter fat, synthetic lard, hydrogenated coconut oil, corn oil, olive oil, hydrogenated sesame oil, sesame oil." Since cottonseed oil and sesame oil are similar in their fatty acid make-up, it is difficult to understand why these oils differ so greatly in their fat sparing action. From a still later study (91) it was found that fat exerted a measurable sparing action upon the vitamin B content of tissues of young rats. The loss of the vitamin B in the feces bore no relation to the loss from the tissues, and therefore it appears that fat spares vitamin B by decreasing the amount of this vitamin dissipated in the metabolism of the rat.

Evans, Lepkovsky and Murphy (92) find that fat exerts no sparing action upon vitamin G.

In concluding this brief discussion, we present the views of Cowgill (93, p. 97) who has long been a student of the physiology of vitamin B. He comments on this controversial topic as follows: "The idea that vitamin B may be present in fat cannot be rejected as completely untenable. It is conceivable that the vitamin can exist in such a chemical form as to render it soluble in fats and materials which dissolve fat. Van Veen—has shown that this antineuritic substance reacts similarly to the better known organic bases, and therefore, should be soluble in organic solvents when present as the free base, and insoluble in these when present as a salt. By an application of this principle Block and Cowgill—succeeded in extracting large amounts of vitamin B from rice polishings and yeast by use of ether and other organic solvents. Such a result was directly contrary to those reported in earlier literature which seemed to prove conclusively that vitamin B is insoluble in ether. In view of these recent developments one is justified in believing that the so-called 'vitamin sparing action of fats' may after all prove to be due to the actual presence of vitamin B in the fat. There are instances in the beri-beri literature where 'fat' was remarkably effective in curing epidemics. Whether the fat actually furnished vitamin B or acted simply to spare this dietary essential is immaterial so far as its value in

curing the epidemic is concerned. If the sparing action, as expressed in terms of 'equivalence of vitamin' was great enough, a cure could readily result."

RELATION OF DIETARY FAT TO BODY FAT. Unlike the uniform tissue proteins and stores of carbohydrate represented by glycogen, the fats of the organism are not always merely synthesized *de novo;* otherwise, we should expect them to be more uniform in character. There is a striking illustration of large economic significance of this fact in animal husbandry in the production of "soft pork." The "soft pork" problem is fundamentally one of fat metabolism. The quantity and quality of the fat in feeds exert a marked influence on the firmness of body fat. The percentage of fat in the more or less common hog feeds varies from less than one per cent to approximately 50 per cent.

Hog carcasses are graded "oily," "soft," or "hard," depending upon the type of diet consumed by the hogs. "The products of soft hogs have certain undesirable characteristics. There is a lack of firmness in the fat which may be so extreme as to cause a typical soft, flaccid, shapeless condition in the products, which makes them inconvenient to handle and unattractive to many people. The hams are probably least and the lard most subject to criticism, depending on the proportion of fat in the product" (94). The undesirable characteristics of the products from "soft" and "oily" hogs place them at a disadvantage on the market with resulting discrimination in live-hog price. Later in this section, reference will be made to investigations on this problem of the meat industry.

The study of the influence of dietary fat on the quality of body fat has engaged the attention of investigators for a long time. More than fifty years ago Lebedeff (95) performed some striking experiments with two dogs which he fasted until each one lost 40 per cent of its original body weight. Subsequently, he fed rations rich in fat until each dog regained its original body weight. The animals were then killed. From the body fat of the dog fed linseed oil, he obtained a large quantity of oil which did not solidify at 0°C.; whereas, from the adipose tissues of the dog fed mutton tallow, a fat "which was almost identical with mutton tallow" was observed. The melting point of mutton tallow is above 50°C. Subotin had previously reported that normal dog fat melts between 30° and 42°C. A little later Munk (96) performed a similar experiment in which he fed rapeseed oil to a dog. From the body fat of this animal he isolated erucic acid which is present in abundance as erucin in rapeseed oil, and concluded, that this constituent

glyceride of the dietary fat had been deposited. This early work of Lebedeff and of Munk and of other investigators including Radziejewsky, Hoffmann and Lummert has been reviewed by Rosenfeld (97), by von Fürth (98) and by Leathes and Raper (99). Mendel (100) reviewed the later literature in his stimulating lectures on the relationship of diet to fat deposition in the body.

The experiments of Lebedeff and Munk were of an extreme type. With the more recent development of the science of nutrition, it became possible to plan experimental feeding under conditions in which a simple factor can be altered at will without profoundly upsetting the proportional relations of the other dietary components. Accordingly Anderson and Mendel (101) who conducted a number of comparative experiments with rats, fed rations in which 60 per cent of the energy intake was variously offered in the form of fat or carbohydrate. When soy bean oil, corn oil, cottonseed oil, peanut oil or "Crisco" (hydrogenated vegetable oil) was ingested the resulting body fat closely resembled the food fat as indicated by a comparison of iodine numbers. However, close similarities between food fat and the body fat did not always develop. The body fat produced when lard, butter fat or coconut oil was consumed, was more unsaturated than the ingested fat. Coconut oil which has a low unsaturated acid content and an iodine number of only 8, produced a body fat with iodine number of 35. Apparently the animal organism desaturated the dietary fat and deposited a body fat which is less saturated than "physiological fat" (iodine number 65) synthesized from carbohydrate. The influence of a number of fats including linseed oil, cod liver oil and olive oil, upon the phospholipids and neutral fat of the body fat of the rat, has been reported also by Sinclair (102). The effect of dietary changes after "soft" fats have been deposited in the body has been studied also by Anderson and Mendel. When carbohydrate (corn starch) was substituted equicalorically for fat in the diet, a progressive "hardening" of the body fat ensued. Presumably a partial depletion or transformation of the fat reserves was accomplished only slowly. The change from one type of specially produced depot fat to another was brought about far more rapidly by depleting the fat reserves through brief periods of fasting prior to the change in the dietary.

From a study of the factors influencing the distribution and character of adipose tissue, Reed, Yamaguchi, Anderson and Mendel (103) found that on equicaloric intakes nearly 65 per cent to 100 per cent more fat was stored by rats ingesting rations rich in fat than by those on a

diet rich in carbohydrate (corn starch). In some of the experiments in this investigation, fat or carbohydrate was fed at the high level of 83 per cent of the total energy intake. The proportional distribution of the intermuscular, genital, subcutaneous, perirenal, mesenteric and omental depot fat was similar for animals of the same sex and body weight, regardless of the diet fed. Fat stored in different depots showed slight variations only in degree of saturation; differences in chemical nature were not coincident with the location of the fat.

In animal husbandry attention has been paid to the question of distribution of fat in the body because of its bearing upon the value of the carcass. Hammond (104) who considers the problem of fattening of farm animals, remarks that fat deposited as marbling (intramuscular) fat is of much greater market value than the same amount of fat deposited in the caul (omentum) or mesentery. The richness in flavor and tenderness of meat which are highly appreciated are due largely to the presence of marbled fat (18).

Extensive coöperative experiments dealing with the "soft pork" problem have been reported (94, 105). These tests were conducted by the United States Department of Agriculture and by a considerable number of State Experiment Stations. The reports referred to contain a review of the earlier work done in this country and in other countries including Canada and Europe. Consideration will now be given to the studies of Ellis and co-workers who actively participated in the aforementioned coöperative experiments.

Ellis and Isbell (70) have made a study involving the complete separation of the fatty acids of samples of fat obtained from hogs fed peanuts, soy beans, corn and skim milk, and brewer's rice and tankage. Whole peanuts and beans have a fat content of 33 and 18 per cent respectively; the corn and skim milk ration contains about 4.5 per cent fat, whereas the ration of brewer's rice and tankage has the very low fat content of one per cent. The body fat produced on the latter diet (low fat) was found to be very "hard." The carcasses of hogs produced on corn and skim milk were also "hard," as evidenced by the iodine number and refractive index values of 53 and 1.4582, respectively. (The refractive index has been used extensively by Ellis and co-workers in measuring saturation and firmness of hog fats.) On the other hand, the hogs reared on peanuts and soy beans—feeds rich in oil—were "soft" and "oily." The iodine number and refractive index of one sample of "soy bean lard" was 101 and 1.4636, respectively. The oils of the peanut and soy bean have a pronounced effect on the composition of the lard.

A greater likeness was noted between peanut oil and "peanut lard" than between soy bean oil and "soy bean lard." Whereas the very "hard" fat had a glyceride of linoleic acid content of about one per cent, one sample of "soy bean lard" contained as high as 38 per cent of this glyceride. Furthermore, in the very "hard" and "soft" fat, the glycerides of saturated fatty acids amounted to 40 per cent and 24 per cent, respectively, of the total glycerides. Linoleic acid has an extreme softening effect. On the other hand, the saturated fatty acids display the opposite effect. In all samples of fat studied "palmitic acid and stearic acid occurred in a ratio of 2:1."

Ellis (106) has also reported on the changes in quantity and composition of fat in hogs fed a peanut ration followed by a corn ration. Some young hogs which gained between 40 pounds and 50 pounds on the peanut ration were subsequently changed to the corn ration during which feeding period there was an average gain in body weight of 150 pounds. As a result of the hardening process the body fats displayed the following changes: the iodine number was reduced from 93 to 63; the saturated fatty acids were increased from 19 per cent to 33 per cent; and the linoleic acid content was reduced from 23 per cent to 9 per cent.

The relation of cottonseed oil in the diet to quality of fat in the hog has also been investigated by Ellis, Rothwell and Pool (107). Corn, peanut, and soy bean oils fed at a 4 per cent level of the ration produced a distinct softening of the body fat whereas cottonseed oil, fed to the same extent, had the opposite effect. When cottonseed oil was added to basal rations at 4, 8 and 12 per cent levels, the hardest carcasses were secured at the 4 per cent level. The higher quantities resulted in increasing softness. Among the striking changes in the fatty acid distribution there was a marked increase in the linoleic and stearic acids at the expense of the oleic and palmitic acids. Although the maximum content of total saturated acids occurred in the 4 per cent level, the maximum in stearic acid increased steadily up to the 12 per cent level; at this level of feeding, the stearic acid content (25 per cent) of the body fat was nearly twice that of the palmitic acid content. "It is suggested that the changes in composition of the lard are due to the presence in cottonseed oil of one or more isomeric forms of oleic or possibly linoleic acid which are converted readily into stearic acid."

Eckstein (108) who fed fat-free diets and diets containing preformed fat, analyzed not only the body fat of the whole rat but also the fat of parts of the body including hide; all organs except the brain; brain; and carcass (residual part of the animal). Eckstein remarks: "A definite

change in the nature of the lipids was obtained as the result of the ingestion of myristic acid, triolein, and sodium butyrate. The butyryl radical was apparently not deposited in the tissues, while the other two were. Arachidonic and linoleic acids were always present in the tissues of the rat, and while the contents of the total lipids were found to vary considerably with the diets the amount of more highly unsaturated acids was appreciably altered only by the feeding of triolein." Concerning the distribution of lipids in the various fractions, the percentage of total fat was found to be more constant in the hides than in the carcasses; and in accordance with the prevailing view the lipids in the organs contained more of the unsaturated acids than did the lipids of other parts of the body. Eckstein confirmed his observation that fatty acids of low molecular weights are not incorporated in the body fat of rats by feeding tricaproin (109) and butter fat (110). Apparently tricaproin and the glycerides of the low fatty acids of butter are transformed into "new fat." "Fatty acids of low molecular weight are probably utilized to synthesize saturated fatty acids with a mean molecular weight similar to that of those fatty acids synthesized from fat-free precursors."

Leube, cited by von Fürth (98), had previously failed to find evidence that the volatile fatty acids of butter fat were deposited in the adipose tissue of a dog. Davis (111) also reports that tributyrin of dietary origin was not stored in the depot fat of the hen. Powell (112) remarks that the general effect of feeding tricaprylin agrees with that reported by Eckstein for the butyryl and caproyl radicals. On the other hand the feeding of trilaurin produced a depot fat which contained as much as 25 per cent lauric acid. The observations that the lower fatty acids are not deposited in body fat led Hughes and Wimmer (113) to test the validity of the assumption that the short-chain acids in butter fat follow the path of absorption of non-lipids during the process of digestion. The results of their experiment which tend to prove that their assumption is valid indicate that there is no increase in the amount of soluble, volatile fatty acids present in the thoracic lymph as glyerides.

Spadola and Ellis (53) compared the influence of feeding cottonseed oil and hydrogenated cottonseed oil with a low fat diet. On the latter dietary regimen myristic, palmitic, stearic, palmitoleic and oleic acids were found in the body fat. Palmitic acid accounted for nearly 30 per cent, oleic acid for almost 50 per cent and palmitoleic acid for 14 per cent of the total fatty acids. Obviously stearic acid, in comparison with palmitic acid, occurred in small proportions. The unsaturated components of the adipose tissue were more greatly influenced by the ingestion

of oil than were the saturated acids. The amount of linoleic acid deposited was approximately proportional to the quantity ingested. This observation is in accord with remarks regarding the occurrence of this fatty acid in the body fat of the hog (52, 70, 114). Increased deposition of linoleic acid was accompanied by a decrease of palmitoleic and oleic acids in the adipose tissue of the rat. Lack of sufficient material had made it impossible for Banks, Hilditch and Jones (115) to obtain decisive evidence regarding the deposition of palmitoleic acid but the finding of this constituent in the body fat of the rat by Spadola and Ellis (53) is confirmation of the work of Klenk, Ditt and Diebold (116). These German investigators reported the presence of this acid in the phospholipid-free fat of rats which had been fed whole barley grain (Gerstenschrot).

Banks and co-workers (115) also reported that body fats of rats produced under various dietary regimens contained 25 to 30 per cent of palmitic acid. The lipids produced on rations containing various levels of cod liver oil contained nearly nine per cent of unsaturated $C_{20,22}$ acids and only four per cent of the less saturated constituent, namely, linoleic acid. In comparing the fat of various species, these workers remark that "the proportions of stearic acid are strikingly different in the body fats of the rat and of pigs, sheep and oxen. In the latter group stearic is definitely a major component acid, occurring in widely varying amounts. In the rat body fats it is equally clearly a minor component and occurs in proportions relatively small compared with those of palmitic or oleic acid." The following comment is offered on this statement: Concerning the prominence of palmitic acid in hog fat, Ellis and Isbell (70) reported the occurrence of palmitic acid and stearic acid in the ratio of 2:1. The fats examined by these investigators were produced by hogs which varied in grade all the way from "hard" to "soft" and "oily." Bhattacharya and Hilditch (117) who compiled many data based on molar distribution of fatty acids in the fat of hogs show the ratio of palmitic acid to stearic acid to range from over 3:1 to below 1.5:1. The data referred to include those obtained by these English investigators on the body fats of hogs reared on rations, some of which contained small amounts of shea butter fat (obtained from the seeds of the shea tree) and peanut oil. It would appear, therefore, on the basis of these remarks, that in hog fat, palmitic acid may be considered more of a "major" component than stearic acid. Apparently palmitic acid is deposited in appreciably larger quantities than stearic acid in the fat of hogs, except as previously noted, under a dietary

regimen which includes cottonseed oil (107). Concerning the distribution of stearic and palmitic acids in mutton and beef, Hilditch (72) presents data to show that in mutton tallow stearic acid exceeds palmitic acid, whereas in beef tallow palmitic acid is deposited largely in excess of stearic acid. In beef tallow palmitic acid would appear therefore to be the "major" of the two acids.

The influence of cereal diets on the body fat of the rat has been reported by Olcott, Anderson and Mendel (118). In this study the cereals, corn, oats, wheat, or barley supplied 82 per cent of the total energy value of the ration and the various cereal oils furnished three per cent to ten per cent of the total calories. Body fats produced on these cereal diets exhibited iodine values ranging from 70 to 83; and the unsaturated fatty acid content was found to range from 68.5 per cent for fat obtained from rats reared on the barley diet to 75 per cent for fat of rats produced on the corn ration. Previous work done by Henriques and Hansen (119) had shown that the iodine value of the fat of hogs fed barley and maize was approximately 58 and 76 respectively.

An interesting study made by Barbour (120) indicates existence of a threshold for saturated acids in the body fat of the rat. He observed that "the saturated acid content of the body fat may be lowered by feeding fats of low saturated acid content, but cannot be raised beyond a level of 25 to 27 per cent by feeding fats of higher saturated acid content. In the latter case the excess saturated acid is excreted in the feces, which may thus have a very high saturated acid content." This limitation in saturation is in marked contrast to the unsaturation of body fat which, as already noted (70, 101), can be influenced tremendously by the presence of fat in the diet. In this connection Barbour remarks that "in the albino rat fed at a 20 per cent level—the unsaturation of the body fat, as measured by the iodine value, is proportional to the unsaturation of the dietary fat."

Inasmuch as large amounts of hydrogenated fats are currently consumed, it is of interest to obtain information regarding the deposition and utilization of iso-oleic acid, a constituent of hardened oils and fats. Barbour (121) has reported such a study in which evidence is adduced that iso-oleic acid, an isomer or mixture of isomers of oleic acid, may serve as a source of energy as satisfactorily as other fatty acid components. Spadola and Ellis (53) also reported that ingested iso-oleic acid was deposited in depot fat. Schoenheimer and Rittenberg (122) who fed mice various amounts of deuterium-containing fat, found an indication that the largest part of the dietary fat is deposited in the fatty tissues before it is utilized.

Studies concerning the influence of dietary fat on fetal fat have yielded results of a conflicting nature. Needham (123) has reviewed the literature and Chaikoff and Robinson (123A) who also summarized previous studies, conducted a large number of feeding experiments with pregnant rats from which fetuses were obtained on the 20th or 21st day following impregnation. Fats which were fed at a 40 per cent level of the diet, ranged in iodine number from 8 to 179. Under these experimental conditions, "although marked variations in the degree of unsaturation of maternal fats were produced as shown by iodine numbers ranging from 36.3 to 145, the iodine values for fetal fat lay within the limits of 61.5 to 103." These observations indicate that as far as high fat diets are concerned, modification of fetal fat may occur within limits. The possibility is also suggested "that transmission of fat through the placenta does occur, and that once within the fetal organism, a qualitative transformation of this fat is effected."

Cruickshank (71) has investigated the effect of ingested fat on the composition of egg fat and of body fat of the hen. When fats which vary in solid (saturated) acids from 9 to 51 per cent are fed at a level of 28 per cent of the ration, eggs are produced which contain not less than 24 per cent and not more than 30 per cent of saturated fatty acids. Apparently "the ingestion of saturated acids had relatively little effect in altering the normal composition of the mixed acids of egg fat." On normal cereal rations, containing protein supplements, the linoleic acid content of egg fat was 15 to 19 per cent, whereas on rations containing oils rich in linoleic acids, egg fat was produced in which this so-called essential fatty acid exceeded 40 per cent of the total mixed fatty acids. Grossfeld (124) reported that egg oil obtained from market eggs of Danish origin contains 18 per cent of linoleic acid. Variations in oleic acid and linolenic acid content of egg fats are indicated also by Cruickshank (71). Concerning the effect of dietary fat on body fat of the hen this investigator who ascertained character of fat by means of the iodine value, interestingly remarks that the body fat is markedly influenced by ingested fatty acids, irrespective of their degree of saturation. "It seems possible also that changes in the composition of the fat can be more rapidly induced in the bird than in the mammal, owing to the higher rate of metabolism of the former and the resulting rapid utilization of food." Cruickshank includes in her report a survey of the earlier studies of fat metabolism in the hen. Hilditch, Jones and Rhead (125) who examined depot fats of hens fed rations containing four per cent or less of fat, reported on the distribution of the fatty acids. On a

weight basis palmitic acid and stearic acid were present in the ratio of 6:1; and the content of palmitoleic, oleic and linoleic acids were 7, 43 and 19 per cent, respectively.

Almquist, Lorenz and Burmester (126) who conducted tests on the relation of depot fat to egg yolk fat of hens, fed crude cottonseed oil, concluded on the basis of the Halpen test, that depot fat is not utilized to any important extent in the formation of yolk fat. This is confirmation of work reported by Henderson and Wilcke (127) who showed that Sudan III pigment is not transferred from the abdominal fat to the yolk fat. This dye appears in eggs three to five days after it is included in the fatty component of the diet.

The foregoing remarks concerning the relation of dietary fat to body fat and to egg fat indicate some of the chemical transformations of fat in the organism, and may serve to emphasize the vigorous investigation currently taking place in this field of lipid chemistry.

RELATION OF DIETARY FAT TO MILK FAT. It was at one time believed possible to "feed fat into milk." In 1866 Stohmann (128) fed goats diets high and low in fat in alternate periods. The percentage of milk obtained with the low fat ration was 30 per cent lower than the percentage of the milk fat secreted during the ingestion of the high fat diet. This study furnished a striking illustration of the influence of diets rich and poor in fat on the fat content of the milk. Stohmann's work has been previously overlooked in the discussion of the influence of fat-poor rations on milk and fat production. Soxhlet's (129) statement in 1896 that "normal butter fat is certainly a product of the activity of the lacteal glands" appears prophetic in the light of the recent work of Petersen, Palmer and Eckles (130) which indicates that the mammary gland of the cow takes a more active part in the formation of milk fat than was heretofore suspected. This investigation shows that the fat of the lactating gland is intermediate in composition between the body fat and butter fat. The fat of the non-lactating gland is similar in composition to body fat.

Maynard and McCay (131) in their summary of the early work on the origin of milk fat from the food have pointed out that the pioneer work of Jordan and co-workers (36, 37) demonstrated the fundamental fact that the food carbohydrate is a source of milk fat. From the large number of studies conducted by Morgan, Beger and Fingerling (132, 133, 134) on sheep and goats it was concluded that, whereas carbohydrate can be utilized to manufacture milk fat, fat itself is a more suitable material, and that within certain limits the percentage of fat

in the milk is influenced by its content in the feed. Maynard and McCay (131) point out that while the differences obtained were fairly consistent they were small in most cases and that the significance of many of the data is questionable because the rations compared differed with respect to their ingredients and also as regards the quality of their protein and other factors.

These experiments among others raised the important question, which has been studied by Maynard and McCay (131, 135), as to whether carbohydrate is equally as satisfactory as fat, from the standpoint of physiological economy and of yield of secreted product, as the food source of milk fat. These investigators have shown clearly that a low-fat diet decreases the milk and fat yields of dairy cows. In one period they fed a typical dairy ration; in an alternate period the same ration in which the fat was extracted from the grain component and replaced by an isodynamically equivalent amount of starch, was fed. Their work indicates that in high yielding cows there is a limiting factor in the synthesis of milk fat from carbohydrate. This limiting factor may be the result of one of two apparent possibilities or of both. First, it may be the result of the inability of the cow to synthesize fat from carbohydrate in sufficient quantities to meet the demands of the lactating gland in which case the cow limits the amount of milk secreted in order to maintain a constant fat percentage of milk. In the second place, the gland in its production of milk fat may require certain specific fatty acids which the animal is unable to synthesize.

The possible need of essential fatty acids in lactation is suggested by the evidence cited in the previous section of this review on Essential Fatty Acids. Bender and Maynard (136) studied this problem in lactating goats by substituting linseed or coconut oil for an equicaloric amount of starch in a low-fat ration (0.45 per cent ether extract). No difference was obtained between the influence of the unsaturated oil and the saturated oil on the milk and fat yields. The limited observations were interpreted to indicate that at least one of the causes of the lowered yields on a fat-poor ration is a lack of fat per se. A further study of this same problem was conducted by Williams and Maynard (137) who also used lactating goats. They employed a more highly purified ration (less than 0.2 per cent ether extract, regenerated cellulose as roughage) as the basal fat-free diet to which was added in different feeding periods butter oil, coconut oil, palmitic and stearic acids, and oleic and linoleic acids. In every case the fat-free ration caused a very rapid drop in milk yield and an equal or greater decline in fat yield.

These declines ranged from 25 to 65 per cent for the various goats from the start to the end of the period. In no case did the inclusion of a fat supplement in the ration have any effect on milk yield beyond arresting wholly or in part the decline on the fat-free ration, although the butter oil and coconut oil increased the fat percentage. At present there is no evidence to postulate the lack of the so-called essential fatty acid as the cause of the decline of milk and fat yields in herbivora on low-fat rations. Rather, the lack of fat per se, appears as the important factor.

The study of Hansen (138) and that of Völtz (139) in feeding coconut oil meal and palm kernel oil meal pointed to a slight increase in the percentage of the milk fat but poor technique obscured the significance of their results. The investigations of Frederiksen (140) with the same oil meals show an increase in fat percentage large enough to offset the decrease in milk yield. These experiments appear to be significant. Confirmatory evidence has been reported by Brouwer (141) and Schmidt and Vogel (142). Perkins (143) obtained negative results with coconut meals whereas the positive results of Honcamp and co-workers (144) were inconsistent. Beger (145) has shown that the feeding of butter fat to goats increased the fat percentages of the milk. Allen (146) has demonstrated a marked increase in the butter fat yield of dairy cows fed whole milk in addition to the regular ration.

Much of the work on the feeding of palm kernel oil, coconut oil and butter fat to lactating animals lacks significance due to the fact that the augmented fat content of the milk resulted from lowered milk yield rather than to the influence of increased dietary fat. The work as a whole, however, indicates a positive effect in increasing yield of fat. Maynard (147) has recently summarized the evidence as follows: "As a result of the many studies of the influence of specific dietary fats on milk fat percentage, two findings stand out—the one, that cod liver oil lowers fat percentage and the other, that feeding butter raises it. The explanation of the latter may lie in the fact that the gland is presented with exactly the same fatty acids which it needs for its secretion. Among the many contradictory and inconclusive studies of the influence of other dietary fats on fat percentage those by European investigators reporting positive effects from coconut oil meal and palm kernel oil meal high in fat are sufficiently consistent as to merit attention. These fats are rather unique as regards fatty acid distribution in containing, as does milk fat, a considerable amount of acids lower than C_{16}, though not containing the very low ones present in milk fat. Since butter itself is

effective, perhaps a fat with a fatty acid distribution approaching it in some respects would have some effect. The recent report of Allen (148) that a high fat diet obtained by the use of a variety of fats uniformly raises fat percentage, at least temporarily, represents more constant and positive results than have previously been reported."

There are numerous reports concerning the influence of feeding cod liver oil to lactating cows. Golding and coworkers (149) found a decrease of about 30 per cent in the fat. Petersen (150) and Hart and coworkers (151) offer confirmation of this result. Golding (152) reported that the non-saponifiable fraction of the oil did not cause the lowering of the milk fat. McCay, Maynard et al. (153) confirm these observations and report further that the injurious fraction is carried by the triglycerides of the cod liver oil. A recent study by Hilditch and Thompson (58) of the fatty acids secreted in the butter fat of cows ingesting various oils (rape oil, linseed oil and cod liver oil) indicates that the causative agent resides in the highly-unsaturated C_{20-22} glycerides which are notably present in cod liver oil. The milk fat produced on the cod liver oil diet contained five to seven per cent of the highly-unsaturated C_{20-22} fatty acids. They suggest that selective adsorption of these highly-unsaturated compounds by the enzymes of the mammary gland, responsible for the elaboration of typical cow milk fats, retards (acts like a "catalyst poison," analogous to an antioxidant) their normal function and causes the observed effect on lowering the percentage of milk fat secreted.

The studies of Gage and Fish (154) indicate that the source of milk fat is different in the carnivorous and omnivorous animals from that of the herbivorous animals. In the carnivorous and omnivorous animals where fat has a relatively important place in the natural diet, the milk fat arises more directly from the fat of the food. As pointed out by Mendel and Daniels (155) and corroborated by Gage and Fish the fat depots are readily drawn upon as a source of milk fat in case of insufficient fat in the food. In the case of herbivorous animals where fat apparently plays a less important part in the natural diet, the milk fat arises to a greater extent from the carbohydrate of the food. Gage and Fish found by their technique (fat soluble dye) that the goat was intermediate between the cow and the rat in its ability to use food fat as a source of milk fat. Commenting on this work and that of Petersen, Palmer, and Eckles (130), Bloor (156) states: "These results indicate that the mammary gland in the cow takes a definite part in the formation of milk fat, which would go to explain the lack of direct connection be-

tween food fat and milk fat in herbivorous animals as compared with the direct connection noted in omnivorous and carnivorous animals." In human lactation studies Macy and coworkers (157) found that milk fat was equivalent to 74, 50 and 43 per cent of the total fat intake of three individuals.

The work of Holland and coworkers (55) and of Bender and Maynard (136) show that the quality of the fat in the diet exerts an effect on the quality of the butter fat, constituting evidence that the food fat is an important source of the milk fat. The more saturated the fat of the food, the lower was the iodine number of the butter fat, and the opposite result occurred when the food fat was more unsaturated. Significant work reported by Brown and Sutton (158) shows the effect of feeding menhaden oil on the milk secretion and the composition of butter fat in the cow. The oil tended to lower milk production, percentage of fat, and total fat yield. Resumption of the feeding of the control diet brought about a slow recovery of normal milk and milk fat production. The characteristic highly unsaturated acids of the fish oil passed in small amounts into the butter fat, the analytical constants of which showed it to be a mixture of butter fat and menhaden oil. Buschmann (159) added vegetable oils to the ration of dairy cows varying from 0.4 to 1.0 kilo of oil per day per 1000 kilos of body weight without obtaining a significant change in fat content of the milk. The chemical and physical constants of the milk fat were altered in such a way as to indicate that approximately 18 per cent of the particular oil had gone directly into the milk fat.

Studies (57, 58, 160) of the dietary influence of linseed oil, rape oil and particularly cod liver oil on the fatty acids of milk fat clearly demonstrate that ingestion of certain oils by lactating cows causes striking modifications of the butter fat secreted. Recently Maynard, McCay and Madsen (161) have reported a study with cows fed in alternate periods, rations containing fats of a high and low degree of unsaturation. In half of the cases a marked change was noted in the iodine number of the milk fat secreted during the first 18 hours following a change in ration, and in all cases within the next 24 hours; the maximum change was attained within three or four days. These data furnish evidence of a close relationship between food fat and milk fat and are opposed to the conclusion of Gage and Fish (154) that in the cow, milk fat is derived only to a small degree from the fat of the food.

In conclusion, it seems evident that in the carnivore and omnivore there is a direct relationship between the food fat and the milk fat.

In the herbivore, this relationship appears less direct. The recent studies demonstrate a markedly rapid effect of the quality of food fat on that of milk fat in the herbivorous animal where carbohydrate has been shown to be an important source of milk fat. In addition it appears evident that below a certain minimum fat intake, the production of milk fat from carbohydrate is limited. The indications are a closer relationship between dietary fat and milk fat which places a stronger emphasis on the importance of food fat as a source of milk fat than was previously recognized.

THE ALLEGED TOXICITY OF COD LIVER OIL. During the last ten years there has appeared a long series of papers by Agduhr and other Scandinavian workers (162, 163, 164) claiming that cod liver oil may exert a harmful action when administered over long periods of time. Agduhr (165) working with mice, rats, rabbits, calves, pigs and dogs described pathologic changes produced by the oil even when given in doses as low as 0.1 cc. per kilo per day. The most serious and characteristic lesion was degeneration and fibrosis of the cardiac musculature. Animals of different species were found to differ in their sensitivity to the toxic action of cod liver oil, rabbits and mice being much more susceptible than rats and dogs. Electrocardiographic examination of the affected animals showed that the conducting tissue was damaged, giving rise to irregularity and branch bundle block. The toxic factor was found to be associated with the saponifiable, or fatty acid, fraction of the oil (166). Agduhr (167) has claimed that the experimental periods reported in the literature were too short to produce the characteristic injuries in the rat which is one of the species least susceptible to the toxic action of cod liver oil. In this connection it is interesting to note that Cox and Roos (168) were not able to demonstrate any lesions in rats fed for 130 days a diet furnishing 78 per cent of the calories in the form of cod liver oil. Reference will be made shortly to long time experiments (unpublished) conducted at Yale University.

Harris and Moore (169) and Bell, Gregory and Drummond (170) have presented evidence indicating that the inclusion in the diet of large amounts of cod liver oil causes retardation of growth of the albino rat. Norris and Church (171) thought that the toxicity was due to nitrogenous bases like isoamylamine and choline, but Bell and coworkers (170) found no experimental basis for this hypothesis. They demonstrated that the subnormal growth was not due to any of the recognized factors of the unsaponifiable portion of cod liver oil but were unsuccessful in isolating the factor responsible for this effect. Madsen, McCay and

Maynard (172) published a series of experiments which suggested that there was a relationship between cod liver oil and muscular degeneration of herbivora fed synthetic diets. The heart was frequently involved, and a fatty liver was a characteristic finding. The substitution of a vitamin A-D concentrate markedly delayed the onset and lessened the severity of the injuries. While the omission of a cod liver oil product from the synthetic diet did not entirely eliminate the ill effects, the addition of cod liver oil to a diet of natural foods produced the characteristic muscle lesions. The authors concluded that both the cod liver oil and some other factor of the synthetic diet were causative agents in the production of the lesions. Previous reference (58) has been made to the alleged influence of the highly unsaturated $C_{20\text{-}22}$ glycerides in cod liver oil on milk fat production. Perhaps these same components of the oil may be responsible for the observed subnormal growth in rats and also for changes in tissues in herbivora.

Along with Bell, Gregory and Drummond, and Norris and Church, other investigators have stated that the addition of a liberal amount of yeast to the diet of rats fed cod liver oil improves rate of growth. However, current investigations in Laboratory of Physiological Chemistry, Yale University (173) do not support the claim for any protective action of yeast. In the case of mice no improvement in growth was noted even when a liberal amount of yeast was added to the diet containing 20 per cent cod liver oil. Concerning tissue lesions, histological studies of heart, liver, kidney and muscle tissue of rats and mice fed large amounts of cod liver oil for approximately one year did not reveal pathological changes which could be interpreted as being caused by the cod liver oil.

DIGESTIBILITY AND ABSORPTION OF FAT. Studies on different animal species, including man, with a variety of dietary fats have presented ample proof that food fat is readily digestible. The early studies in this field have been ably reviewed by Bloor (174, 175). Langworthy (176) has reported an extensive series of experiments in which tests of digestibility were made of oils and fats including hydrogenated fats. The results indicated that most of the common fats which are utilized 93 to 98 per cent by the human body, can be readily tolerated in sufficient quantity to furnish more than 1000 calories, or more than one-third of the average energy consumption. It was also indicated that the digestibility coefficient of fats with melting points above body temperature, (37°C.) varies inversely with the melting point. Krakower (177) observed complete absorption in humans who consumed fats of a widely

different character and in amounts which varied enormously (8 gm. and 128 gm. daily). Levine and Smith (178) reported that rats fed fat which furnished 86 per cent of the total energy intake utilized 98 to 99 per cent of ingested fat.

Holt and coworkers (179) have written an extensive report based on studies of various factors which influence fat absorption in normal infants. They believe that barring conditions of low fat intake the most significant measure of fat absorption is the percentage of fat retained. In feeding tests with homogenized cow's milk, and with unemulsified butter in cow's milk, it was shown that the size of fat particles is without influence on fat absorption. In this connection it is recalled that indigestibility of cow's milk as compared with breast milk has been attributed to the alleged larger globules of fat. Volatile fatty acids fed freely in the form of butter with added tributyrin and free volatile fatty acids were found to be well absorbed. The influence of length of fatty acid chain upon absorption was also studied. Two highly saturated fats, coconut oil and a 2:1 mixture of tripalmitin and tristearin (iodine number 2) were compared. The former fat (iodine number 8) contains a considerable proportion of fatty acids with only 12 and 14 carbon atoms, whereas tripalmitin and tristearin are composed of fatty acids of 16 and 18 carbon atoms, respectively. The results, which confirm earlier work, show that the shorter chain fatty acids were more completely absorbed. Limited evidence is also presented to show that the chemical constitution of the fat rather than its melting point is the important factor in fat absorption. Partially hydrogenated corn oil (melting point 52°C. and iodine number 50), and a tripalmitin-tristearin mixture (melting point 52°C. and iodine number 2) were fed, with the result that the retention of the hydrogenated product was 83 per cent as compared with only 62 per cent for the saturated fat mixture. On the basis of this observation the comment is made that "contrary to the general belief the melting point of a fat is not *per se* the factor determining absorption." Lyman (180) who observed great differences in the absorption of glyceryl stearate and glyceryl palmitate in the dog, has also questioned whether absorption of fat is entirely dependent on melting point.

Holt and coworkers who extended their studies to include a comparison of the absorption of various natural fats and proprietary fat mixtures designed for infant feeding, remark that "the differences in retention—with most of these fats were relatively small and for the normal subject could have but little practical significance." With respect to peanut oil, the statement is made: "Although clinical experiences with peanut

oil appear to have been favorable, evidence from experimental animals suggests that these long chain acids may not be readily disposed of by the body and may accumulate in the liver, a fact which leads one to question the advisability of giving a diet rich in peanut oil for a prolonged period of time." For evidence in support of this statement, reference is made to a number of studies of the lipids of livers. Examination of these various studies shows that in most of them the dietary history of the animals from which the livers were obtained for investigation is not indicated; furthermore, mention of the inclusion of peanut oil in any of the dietary regimens is not made. Barbour (120) who has fed peanut oil to rats "at a level of 20 per cent in a diet otherwise normal," did not find an accumulation of fat in the liver. In the same study it was also shown that arachidic acid of peanut oil is almost quantitatively excreted.

Tidwell et al. (181) confirmed previous observations that premature infants and twins have marked difficulty in fat absorption. In such infants it was found that olive oil and soy bean oil are better absorbed than butter fat.

Steenbock and collaborators (182) have determined the rate of absorption of different fats in rats. The technic (183) adopted for measuring absorption involved the feeding of a definite quantity of fat and subsequent analysis of the intestinal residues. Concomitant use of the chylomicron method and the hemolipokrit method for determining blood fat did not give satisfactory results according to observations of these workers. The percentage of fat absorbed from the alimentary canal of rats was determined at various intervals—2 to 12 hours—after feeding a definite quantity (1.5 c.c.) of fat. Butter oil, halibut liver oil, and cod liver oil were absorbed uniformly at a more rapid rate than lard, corn oil, or partially hydrogenated fats. The rate of absorption of butter fat falls between these two groups. There was some indication that the quantity of unsaponifiable matter present in halibut liver oil and in cod liver oil was partly responsible for the lag in the absorption of these oils in comparison with butter oil. Many of the examined fats did not vary greatly in their absorption. There was, however, in a four hour test period a very decided difference in the rate of absorption of olive oil (63 per cent) and oleo stock (36 per cent). In setting forth their results, Steenbock and coworkers state that they did not attempt to determine whether or not rapidity of absorption is physiologically advantageous.

In a survey of the literature Verzár (184) has ably presented the facts

and the gaps in our knowledge regarding the absorption of fats. Detailed consideration is given to the rôle of lipase and of bile and a brief discussion is presented of the pathological disturbances of fat absorption. From his most recent studies with rats (185, 186) fat absorption appears to be dependent on an active process in the intestinal mucosa which synthesizes the fatty acids to phospholipids and neutral fats, under the influence of the adrenal cortex. In the absence of the hormone the fatty acids diffuse freely into the cells but are not synthesized to neutral fat in the intestinal epithelium.

FAT IN THE DIABETIC DIET. The "cream" which appears in the blood of diabetics led early investigators to think that in diabetes not only carbohydrate but also fat metabolism was impaired. The discovery of insulin made it possible to draw a distinction between the lipemia of the diabetic in acidosis and that seen in diabetes without acidosis. Man and Peters (187, 188) have shown that the lipemia of diabetic acidosis is transitory and is related chiefly to the dehydration of the acidosis.

The problem of the amount of fat in the diabetic diet is still unsettled. Allen's starvation treatment of diabetes and the Petren and the Newburgh and Marsh (189, 190) high fat diets are of historical interest in this connection. Peters at the present time gives to the average diabetic 125 to 175 grams of fat daily which represents 50 to 70 per cent of the total calories (191). Joslin, an enthusiast for undernutrition of the diabetic, writes that he does not think the fat in the diet of his hospital patients within the last year "has been permanently below 50 grams or over 120 grams" (192, p. 261). "The average patient based upon a group of 30 selected cases treated at the George F. Baker clinic recently, was found to be on a diet of carbohydrate 149 grams, protein 70 grams and fat 83 grams" (192, p. 258). In this diet, fat furnished 46 per cent of the total calories. Rabinowitch (193) who has advocated high carbohydrate, low fat diets recommends 50 to 55 grams of fat which, in the diets of ten typical patients, represents 28 per cent of the total calories. Other sponsors, cited by Man and Peters (194), of the high carbohydrate, low fat diets give slightly different proportions of fat.

In this review article it is impossible to summarize all the scientific and clinical data which form the basis for these diverse diabetic diets. Peters (191), in his treatment of the diabetic, feels that the individual as a whole must be considered. A diet adequate in calories is most conducive to general health, to resistance to infectious diseases and to an efficient output of energy. To determine whether the diabetic is pecul-

iar in his absorption of fat, twenty experiments on alimentary lipemia were conducted with 14 diabetics, fed 125 to 175 grams of fat daily. With one exception, the alimentary lipemia in diabetes differed neither in kind nor degree from that of normals and therefore the experiments presented no evidence for the restriction of fat in the diet (195).

Undernutrition with some limitation of caloric intake has been advocated by Joslin because of a statistical relationship between diabetes and obesity (192, p. 61). Such statistical correlation cannot be taken lightly as evidence of cause and effect. In formulating diets for the treatment of diabetes, it must be remembered that it is a wasting disease and the dangers of undernutrition with accompanying increased susceptibility to infectious diseases such as tuberculosis cannot be minimized (194, 196, 192, p. 129). The question may be raised whether the low cholesterol values which Joslin considers indicative of bad prognosis are not referable merely to malnutrition.

Rabinowitch in recommending the high carbohydrate, low fat diet has expounded the arguments that "excess blood cholesterol is an important etiological factor in the production of arteriosclerosis in the young diabetic" (197) and that the blood cholesterol is lower on a high carbohydrate, low fat diet. Other authors, cited by Man and Peters (194), present a variety of opinions in regard to the relation of cholesterolemia and arteriosclerosis. Man and Peters (194) in their study of 79 diabetics, "found severe arteriosclerosis with or without hypertension evident in patients with serum cholesterol normal, below normal and above normal." Furthermore, the evidence that the amount of fat in the diet determines the level of serum cholesterol has not been substantiated by Bruger and Poindexter (198), Curtis, Sheldon and Eckstein (199), or by Man and Peters (194).

A second argument advanced by Rabinowitch (200) that the low fat, high carbohydrate diet increases the carbohydrate tolerance of the diabetic has been investigated also by Barach (201), Rosenberg (202), Czoniczer and Kolta (203), and Geyelin (204). Rabinowitch (193) studied 10 patients for whom he had prescribed first his old diet with 140 to 150 grams of fat and subsequently his new high carbohydrate diet with only 56 grams of fat. The insulin requirements of seven of these patients decreased on the new diet but all of them received 100 to 260 fewer total calories on the new diet than on the old. Of the two patients who received equicaloric diets containing different amounts of carbohydrate and fat, one required 20 units of insulin on each diet and the other required five more units on the new low fat high carbohydrate

diet. Geyelin's claim that on high carbohydrate diets, the insulin requirement per gram of carbohydrate diminishes, is of doubtful significance. The absolute amounts of insulin prescribed by him for eight patients rose in three and fell in three instances. MacBryde (205) in studying different types of diabetes has found that insulin requirements are only occasionally reduced by the use of a high carbohydrate low fat diet.

The diabetic, like any normal individual, for his general well-being and his resistance to infectious diseases depends upon an adequate and well balanced diet. In the light of present knowledge a liberal quantity of fat in this diet appears justified.

REFERENCES

(1) Sinclair, R. G. Physiol. Review **14**: 351, 1934.
(2) Bills, C. W. Physiol. Review **15**: 1, 1935.
(3) Chibnall, A. C. and H. J. Channon. Biochem. J. **23**: 176, 1929.
(4) Horwitt, M. K., G. R. Cowgill and L. B. Mendel. J. Nutrition **12**: 255, 1936.
(5) Prout, W. Chemistry, meteorology and the function of digestion considered with reference to natural theology. Bridgewater Treatise VIII, Pickering, London, 1834.
(6) Report on the Physiological Bases of Nutrition by the Technical Commission appointed by the Health Commission. Series of League of Nations Publications, III Health, 1935. III. 6.
(7) Burnet, E. and W. R. Aykroyd. League of Nations. Quart. Bull. Health Organization **4**: 323, 1935.
(8) Starling, E. H. Brit. Med. J. **2**: 105, 1918.
(9) von Müller, F. N. Y. Acad. Med. Bull. Second Series, **2**: 502, 1926.
(10) Halliburton, W. D. Editor, Physiology and National Needs. Dutton and Co., New York, 1918.
(11) Fats and fatty acids as food. By a Committee of the Royal Society. (5 papers.) J. Physiol. **52**: 328, 330, 333, 339, 344, 1919.
(12) Lepkovsky, S., R. A. Ouer and H. M. Evans. J. Biol. Chem. **108**: 431, 1935.
(13) McClendon, J. F. J. A. M. A. **106**: 64, 1936.
(14) Christensen, E. H., A. Krogh and J. Lindhard. League of Nations. Quart. Bull. Health Organization **3**: 388, 1934.
(15) Murlin, J. R. J. A. M. A. **71**: 950, 1918.
(16) Rose, M. S. The foundations of nutrition. Revised edition, The Macmillan Company, New York, 1933.
(17) Worker's Nutrition and Social Policy. International Labour Office, Geneva. Studies and Reports, Series B (Social and Economic Conditions) no. 23 (1936).
(18) Howe, P. E. Oil and Soap **12**: 4, 1935.
(19) Hamill, J. M. Nutrition Ab. Rev. **3**: 1, 1933–34.

(20) Rose, M. S. J. Home Econ. **17:** 372, 442, 1925.
(21) v. Tyszka, C. Klin. Wchnschr. **13:** 1169, 1934.
(22) Die deutsche Volksernahrung und der englishche Aushungerungsplan. Ed. by P. Eltzbacher (1914), Brunswick, Germany. Transl. into English by S. R. Wells and published by University of London Press under the title: Germany's food: can it last? 1915.
(23) Pearl, R. The nation's food. Saunders Company, Philadelphia, 1920.
(24) Pearl, R. J. Ind. Eng. Chem. **10:** 927, 1918.
(24a) Morey, N. B. Nutrition Ab. Rev. **6:** 1, 1936.
(25) McCollum, E. V. and M. Davis. J. Biol. Chem. **15:** 167, 1913.
(26) Osborne, T. B. and L. B. Mendel. J. Biol. Chem. **17:** 401, 1914.
(27) Osborne, T. B. and L. B. Mendel. J. Biol. Chem. **16:** 423, 1913–14.
(28) Bloch, C. E. J. Hyg. **19:** 283, 1920–21.
(29) The Pharmacopoeia of the United States of America. Eleventh Decennial Revision. Mack Printing Company, Easton, Penn., 1935.
(30) Karrer, P. Chem. Rev. **14:** 17, 1934.
(31) Askew, F. A., R. B. Bourdillon, H. M. Bruce, R. K. Callow, J. St. L. Philpot and T. A. Webster. Proc. Roy. Soc., London, Series B, **109:** 488, 1931–32.
(32) Windaus, A., O. Linsert, A. Lüttringhaus and G. Weidlich. Ann. **492:** 226, 1931–32.
(33) Olcott, H. S. and H. A. Mattill. J. Biol. Chem. **104:** 423, 1934.
(34) Evans, H. M., O. H. Emerson and G. A. Emerson. J. Biol. Chem. **113:** 319, 1936.
(35) Lawes, J. B. and J. H. Gilbert. J. Anat. Physiol. **11:** 577, 1877.
(36) Jordan, W. H. and C. G. Jenter. New York Agric. Expt. Sta. Bull. **132:** 1897.
(37) Jordan, W. G., C. G. Jenter and F. D. Fuller. New York Agric. Expt. Sta. Bull. **197:** 1901.
(38) Osborne, T. B. and L. B. Mendel. J. Biol. Chem. **45:** 145, 1920–21.
(39) Evans, H. M. and G. O. Burr. Proc. Soc. Exper. Biol. and Med. **25:** 390, 1928.
(40) McAmis, A. J., W. E. Anderson and L. B. Mendel. J. Biol. Chem. **82:** 247, 1929.
(41) Burr, G. O. and M. M. Burr. J. Biol. Chem. **82:** 345, 1929.
(42) Burr, G. O. and M. M. Burr. J. Biol. Chem. **86:** 587, 1930.
(43) Burr, G. O., M. M. Burr and E. S. Miller. J. Biol. Chem. **97:** 1, 1932.
(44) Evans, H. M. and S. Lepkovsky. J. Biol. Chem. **96:** 143, 157, 1932.
(45) Evans, H. M. and S. Lepkovsky. J. Biol. Chem. **99:** 231, 1932–33.
(46) Evans, H. M., S. Lepkovsky and E. A. Murphy. J. Biol. Chem. **106:** 431, 441, 445, 1934.
(47) Borland, V. G. and C. M. Jackson. Arch. Path. **11:** 687, 1931.
(48) Sinclair, R. G. Proc. Soc. Exper. Biol. and Med. **27:** 1059, 1930.
(49) Tange, U. Sci. Papers Inst. Phys. Chem. Research (Tokyo) **20:** 13, 1932; Chem. Abst. **27:** 1664, 1933.
(50) Becker, J. Mezogazdasagi Kutatasok, **6:** 363 1933; Chem. Abst. **28:** 2394, 1934.

(51) SAHASHI, U. Sci. Papers Inst. Phys. Chem. Research (Tokyo) **23**: 264, 1934; Chem. Abst. **28**: 3448, 1934.
(52) GREEN, T. G. AND T. P. HILDITCH. Biochem. J. **29**: 1564, 1935.
(53) SPADOLA, J. M. AND N. R. ELLIS. J. Biol. Chem. **113**: 205, 1936.
(54) HILDITCH, T. P. AND J. J. SLEIGHTHOLME. Biochem. J. **24**: 1098, 1930.
(55) HOLLAND, E. B., M. E. GARVEY, H. H. PIERCE, A. C. MESSER, J. A. ARCHIBALD AND C. O. DUNBAR. J. Agric. Research **24**: 365, 1923.
(56) BOSWORTH, A. W. AND J. B. BROWN. J. Biol. Chem. **103**: 115, 1933.
(57) ECKSTEIN, H. C. J. Biol. Chem. **103**: 135, 1933.
(58) HILDITCH, T. P. AND H. M. THOMPSON. Biochem. J. **30**: 677, 1936.
(59) HUME, E. M. AND H. H. SMITH. Biochem. J. **25**: 300, 1931.
(60) GREGORY, E. AND J. C. DRUMMOND. Ztschr. Vitaminforschung **1**: 257, 1932.
(61) BURR, G. O. AND W. R. BROWN. Proc. Soc. Exper. Biol. and Med. **30**: 1349, 1932–33.
(62) WESSON, L. G. J. Biol. Chem. **73**: 507, 1927.
(63) WESSON, L. G. AND G. O. BURR. J. Biol. Chem. **91**: 525, 1931.
(64) BURR, G. O. AND A. J. BEBER. J. Biol. Chem. **97**: proc. xxxvi, 1932.
(65) WESSON, L. G. AND F. C. MURRELL. Proc. Soc. Exper. Biol. and Med. **31**: 1118, 1933–34.
(66) DRUMMOND, J. C. The Harvey Lectures, Series **28**: 202, 1932–33. The Williams & Wilkins Company, 1934.
(67) WILLIAMS, H. H. AND W. E. ANDERSON. Oil and Soap **12**: 42, 1935.
(68) SCHOENHEIMER, R. AND D. RITTENBERG. J. Biol. Chem. **113**: 505, 1936.
(69) JAMIESON, G. S. Vegetable fats and oils. The Chemical Catalog Company, Inc., New York, 1932.
(70) ELLIS, N. R. AND H. S. ISBELL. J. Biol. Chem. **69**: 239, 1926.
(71) CRUICKSHANK, E. M. Biochem. J. **28**: 965, 1934.
(72) HILDITCH, T. P. J. Soc. Chem. Ind. **54**: 139, 163, 184, 1935.
(73) DURKEE, M. M. Ind. Eng. Chem. **28**: 898, 1936.
(74) HANSEN, A. E. Proc. Soc. Exper. Biol. and Med. **30**: 1198, 1932–33.
(75) HANSEN, A. E. Proc. Soc. Exper. Biol. and Med. **31**: 160, 1933–34.
(76) CORNBLEET, T. AND E. R. PACE. Arch. Dermat. and Syph. **31**: 224, 1935.
(77) TAUB, S. J. AND S. J. ZAKON. J. A. M. A. **105**: 1675, 1935.
(78) EVANS, H. M. AND S. LEPKOVSKY. Science **68**: 298, 1928.
(79) EVANS, H. M. AND S. LEPKOVSKY. J. Biol. Chem. **83**: 269, 1929.
(80) EVANS, H. M. AND S. LEPKOVSKY. J. Biol. Chem. **96**: 165, 1932.
(81) EVANS, H. M. AND S. LEPKOVSKY. J. Biol. Chem. **96**: 179, 1932.
(82) EVANS, H. M. AND S. LEPKOVSKY. J. Biol. Chem. **99**: 235, 1932–33.
(83) KEMMERER, A. R. AND H. STEENBOCK. J. Biol. Chem. **103**: 353, 1933.
(84) DRUMMOND, J. C. Lane Medical Lectures: Biochemical studies of nutritional problems. Medical Sciences **3**: no. 2, Stanford University Press, Stanford University, Calif., 1934.
(85) SURE, B. Proc. Soc. Exper. Biol. and Med. **30**: 622, 1932–33.
(86) GUHA, B. C. Biochem. J. **25**: 1367, 1931.
(87) WESTENBRINK, H. G. K. Acta Brev. Neerland. Physiol. Microbiol. **3**: 95, 1933; Chem. Abst. **28**: 7319, 1934.

(88) WESTENBRINK, H. G. K. Arch. Neerland Physiol. **19:** 94, 1934; Chem. Abst. **28:** 3448, 1934.
(89) EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY. J. Biol. Chem. **107:** 429, 1934.
(90) EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY. J. Biol. Chem. **107:** 439, 1934.
(91) EVANS, H. M. AND S. LEPKOVSKY. J. Biol. Chem. **108:** 439, 1935.
(92) EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY. J. Biol. Chem. **107:** 443, 1934.
(93) COWGILL, G. R. The vitamin B requirement of man. Yale University Press, New Haven Conn., 1934.
(94) HANKINS, O. G., N. R. ELLIS AND J. H. ZELLER. Some results of soft pork investigations, II. U. S. Dept. Agric., Dept. Bull. no. 1492, 1928.
(95) LEBEDEFF, A. Centr. Med. Wissensch. **20:** 129, 1882.
(96) MUNK, I. Virchow's Arch. **95:** 407, 1884.
(97) ROSENFELD, G. Ergebn. Physiol. **1:** Section 1, Biochemie, 651, 1902.
(98) VON FÜRTH, O. The problems of physiological and pathological chemistry of metabolism. Transl. by A. J. Smith. J. B. Lippincott Company Philadelphia, 1916.
(99) LEATHES, J. B. AND H. S. RAPER. The fats. 2nd ed. Longmans, Green and Co. London and New York, 1925.
(100) MENDEL, L. B. AND W. E. ANDERSON. Yale J. Biol. and Med. **3:** 107, 1930.
(101) ANDERSON, W. E. AND L. B. MENDEL. J. Biol. Chem. **76:** 729, 1928.
(102) SINCLAIR, R. G. J. Biol. Chem. **92:** 245, 1931.
(103) REED, L. L., F. YAMAGUCHI, W. E. ANDERSON AND L. B. MENDEL. J. Biol. Chem. **87:** 147, 1930.
(104) HAMMOND, J. Growth and the development of mutton qualities in the sheep. Oliver and Boyd, Edinburgh, 1932.
(105) HANKINS, O. G. AND N. R. ELLIS. Some results of soft pork investigations. U. S. Dept. Agric., Dept. Bull. no. 1407, 1926.
(106) ELLIS, N. R. Changes in quantity and composition of fat in hogs fed a peanut ration followed by a corn ration. U. S. Dept. Agric., Tech. Bull. no. 368, 1933.
(107) ELLIS, N. R., C. S. ROTHWELL AND W. O. POOL. J. Biol. Chem. **92:** 385 1931.
(108) ECKSTEIN, H. C. J. Biol. Chem. **81:** 613, 1929.
(109) ECKSTEIN, H. C. J. Biol. Chem. **84:** 353, 1929.
(110) ECKSTEIN, H. C. Proc. Soc. Exper. Biol. and Med. **27:** 419, 1930.
(111) DAVIS, R. E. J. Biol. Chem. **88:** 67, 1930.
(112) POWELL, M. J. Biol. Chem. **89:** 547, 1930.
(113) HUGHES, R. H. AND E. J. WIMMER. J. Biol. Chem. **108:** 141, 1935.
(114) ELLIS, N. R. AND O. G. HANKINS. J. Biol. Chem. **66:** 101, 1925.
(115) BANKS, A., T. P. HILDITCH AND E. C. JONES. Biochem. J. **27:** 1375, 1933.
(116) KLENK, E., F. DITT AND W. DIEBOLD. Hoppe-Seyler's Ztschr. Physiol. Chem. **232:** 54, 1935.
(117) BHATTACHARYA, R. AND T. P. HILDITCH. Biochem. J. **25:** 1954, 1931.
(118) OLCOTT, H. S., W. E. ANDERSON AND L. B. MENDEL. J. Nutrition **10:** 517, 1935.

(119) HENRIQUES, V. AND C. HANSEN. Skand. Arch. Physiol. **11**: 151, 1901–02.
(120) BARBOUR, A. D. J. Biol. Chem. **106**: 281, 1934.
(121) BARBOUR, A. D. J. Biol. Chem. **101**: 63, 1933.
(122) SCHOENHEIMER, R. AND D. RITTENBERG. J. Biol. Chem. **111**: 175, 1935.
(123) NEEDHAM, J. Chemical embryology. Vol. 2, 1190. The Macmillan Company, New York, 1931.
(123a) CHAIKOFF, I. L. AND A. ROBINSON. J. Biol. Chem. **100**: 13, 1933.
(124) GROSSFELD, J. Ztschr. Untersuch. Lebensm. **65**: 311, 1933.
(125) HILDITCH, T. P., E. C. JONES AND A. J. RHEAD. Biochem. J. **28**: 786, 1934.
(126) ALMQUIST, H. J., F. W. LORENZ AND B. R. BURMESTER. J. Biol. Chem. **106**: 365, 1934.
(127) HENDERSON, E. W. AND H. S. WILCKE. Poultry Science **12**: 266, 1933; Nutrition Abst. and Rev. **3**: 880, 1933–34.
(128) STOHMANN, F. J. f. Landw., II, **4**: 116, 1869.
(129) SOXHLET, F. Wochembld, Landw. Ver. Boyern, Separate No. 40, 1896; Exper. Sta. Record **8**: 939, 1016, 1896–97.
(130) PETERSEN, W. E., L. S. PALMER AND C. H. ECKLES. Am. J. Physiol. **90**: 582, 1929.
(131) MAYNARD, L. A. AND C. M. MCCAY. J. Nutrition **2**: 67, 1929.
(132) MORGAN, A., C. BEGER AND G. FINGERLING. Landw. Vers. Sta. **61**: 1, 1904.
(133) MORGAN, A., C. BEGER AND G. FINGERLING. Landw. Vers. Sta. **62**: 251, 1905.
(134) MORGAN, A., C. BEGER AND G. FINGERLING. Landw. Vers. Sta. **64**: 93, 1906.
(135) MAYNARD, L. A. AND C. M. MCCAY. Cornell Univ. Agric. Expt. Sta. Bull. 543, 1932.
(136) BENDER, R. C. AND L. A. MAYNARD. J. Dairy Sci. **15**: 242, 1932.
(137) WILLIAMS, H. H. AND L. A. MAYNARD. J. Dairy Sci. **17**: 223, 1934.
(138) HANSEN, J. Landw. Jahrb. **47**: 1, 1914.
(139) VÖLTZ, W., et al., Landw. Jahrb. **47**: 573, 1915.
(140) FREDERIKSEN, L. Meddelalse fra Forsogslaboratoriets Husdyrbrngsafdeling **1**: 19, 1923; **2**: 20, 1924; **3**: 20, 27, 1927.
(141) BROUWER, E. Verslag, landb. Onderzoek. Rijkslandbouwproefsta. **35**: 39, 1930; **36**: 64, 1930; Chem. Abst. **26**: 1041, 1932.
(142) SCHMIDT, J. AND H. VOGEL. Biedermann's Zentralbl. B. Tierernahr. **3**: 520, 1932.
(143) PERKINS, A. E. Ohio Agric. Expt. Sta. Bimonthly Bull. **152**: 175, 1931.
(144) HONCAMP, F., W. HELMS, G. KODDER AND A. PETERMAN. Milchwirtschaft. Forsch. **9**: 161, 1929; Chem. Abst. **25**: 1004, 1931.
(145) BEGER, C. Landw. Vers. Sta. **64**: 249, 1906; **67**: 1, 1907.
(146) ALLEN, N. N. J. Dairy Sci. **15**: 132, 1932.
(147) MAYNARD, L. A. Proc. Am. Soc. Animal Production, November 29–30, 1935, p. 263.
(148) ALLEN, N. J. Dairy Sci. **17**: 379, 1934.
(149) GOLDING, J., K. M. SOAMES AND S. S. ZILVA. Biochem. J. **20**: 1306, 1926.
(150) PETERSEN, W. E. J. Dairy Sci. **15**: 283, 1932.

(151) HART, E. B., F. B. HADLEY AND G. C. HUMPHREY. Wisconsin Agric. Expt. Sta. Res. Bull. 112, 1932.
(152) GOLDING, J. Proc. World's Dairy Cong. Great Britain 44, 1928.
(153) MCCAY, C. M. AND L. A. MAYNARD (with the coöperation of G. DAVIS, A. HODSON, L. L. MADSEN AND A. ARMITAGE). J. Biol. Chem. **109**: 29, 1935.
(154) GAGE, S. H. AND P. A. FISH. Am. J. Anat. **34**: 1, 1924.
(155) MENDEL, L. B. AND A. L. DANIELS. J. Biol. Chem. **13**: 71, 1912–13.
(156) BLOOR, W. R. Annual Review of Biochemistry **1**: 267, 1932. Stanford University Press, Stanford University, Cal.
(157) SHUKERS, C. F., I. G. MACY, B. NIMS, E. DONELSON AND H. A. HUNSCHER. J. Nutrition **5**: 127, 1932.
(158) BROWN, J. B. AND T. S. SUTTON. J. Dairy Sci. **14**: 125, 1931.
(159) BUSCHMANN, A. Tierernähr. **1**: 129, 1930.
(160) DANN, W. J., T. MOORE, R. G. BOOTH, J. GOLDING AND S. K. KON. Biochem. J. **29**: 138, 1935.
(161) MAYNARD, L. A., C. M. MCCAY AND L. L. MADSEN. J. Dairy Sci. **19**: 49, 1936.
(162) AGDUHR, E. Acta Paediatrica **5**: 319, 1926.
(163) SLAVGOLD, L. Norsk. vet. tidsskr. **6**: 161, 1925; Abst. in Amer. Vet. Med. Assoc. **68**: 236, 1925.
(164) WAHLIN, B. Acta Med. Scand. **74**: 430, 1931.
(165) AGDUHR, E. Acta Paediatrica **6**: 165, 1927.
(166) AGDUHR, E. AND N. STRENSTROM. Acta Paediatrica **8**: 493, 1929; **9**: 229, 1929; **10**: 167, 1930.
(167) AGDUHR, E. Ztschr. Vitaminforschung **3**: 99, 1934.
(168) COX, W. M., JR. AND A. J. ROOS. Bull. Johns Hopkins Hosp. **54**: 430, 1934.
(169) HARRIS, L. J. AND T. MOORE. Biochem. J. **22**: 1461, 1928.
(170) BELL, M. E., E. GREGORY AND J. C. DRUMMOND. Ztschr. Vitaminforschung. **2**: 161, 1933.
(171) NORRIS, E. R. AND A. E. CHURCH. J. Biol. Chem. **89**: 437, 1930.
(172) MADSEN, L. L., C. M. MCCAY AND L. A. MAYNARD. Cornell Univ. Agric. Expt. Sta. Mem. 178, June 1935.
(173) BURACK, E. Private communication.
(174) BLOOR, W. R. The Harvey Lectures, Series 19, 39, 1923–24. J. B. Lippincott Company, Philadelphia, 1925.
(175) BLOOR, W. R. Chem. Rev. **2**: 243, 1925.
(176) LANGWORTHY, C. F. Ind. Eng. Chem. **15**: 276, 1923.
(177) KRAKOWER, A. Am. J. Physiol. **107**: 49, 1934.
(178) LEVINE, H. AND A. H. SMITH. J. Biol. Chem. **72**: 223, 1927.
(179) HOLT, L. E., JR., H. C. TIDWELL, C. M. KIRK, D. M. CROSS, AND S. NEALE. J. Pediatrics **6**: 427, 1935.
(180) LYMAN, J. F. J. Biol. Chem. **32**: 7, 1917.
(181) TIDWELL, H. C., HOLT, L. E., JR., H. L. FARROW AND S. NEALE. J. Pediatrics **6**: 481, 1935.
(182) STEENBOCK, H., M. H. IRWIN AND J. WEBER. J. Nutrition **12**: 103, 1936.
(183) IRWIN, M. H., H. STEENBOCK AND V. M. TEMPLIN. J. Nutrition **12**: 85, 1936.

(184) VERZÁR, F. Nutrition Ab. and Rev. **2**: 441, 1932–33.
(185) VERZÁR, F. J. Physiol. **84**: 41P, 1935.
(186) VERZÁR, F. AND L. JEKER. Pflüger's Arch. **237**: 14, 1936.
(187) MAN, E. B. AND J. P. PETERS. J. Clin. Invest. **12**: 1031, 1933.
(188) MAN, E. B. AND J. P. PETERS. J. Clin. Invest. **13**: 237, 1934.
(189) NEWBURGH, L. H. AND P. L. MARSH. Arch. Internal Med. **26**: 647, 1920.
(190) NEWBURGH, L. H. AND P. L. MARSH. Arch. Internal Med. **31**: 455, 1923.
(191) J. P. PETERS. Personal communication.
(192) JOSLIN, E. P. The treatment of diabetes mellitus. 5th ed. Lea and Febiger, Philadelphia, 1935.
(193) RABINOWITCH, I. M. Canad. M. A. J. **23**: 489, 1930.
(194) MAN, E. B. AND J. P. PETERS. J. Clin. Inves. **14**: 579, 1935.
(195) MAN, E. B. Personal communication.
(196) MAN, E. B. AND E. F. GILDEA. J. Clin. Investigation **15**: 203, 1936.
(197) RABINOWITCH, I. M. Ann. Internal Med. **8**: 1436, 1934–35.
(198) BRUGER, M. AND C. A. POINDEXTER. Arch. Internal Med. **53**: 423, 1934.
(199) CURTIS, A. C., J. M. SHELDON AND H. C. ECKSTEIN. Am. J. Med. Sci. **186**: 548, 1933.
(200) RABINOWITCH, I. M. Canad. M. A. J. **28**: 162, 1933.
(201) BARACH, J. H. J. A. M. A. **98**: 1265, 1932.
(202) ROSENBERG, M. Deutsch. med. Wchnschr. **59**: 320, 1933.
(203) CZONICZER, G. AND E. KOLTA. Med. Klin. **28**: 752, 1932.
(204) GEYELIN, H. R. J. A. M. A. **104**: 1203, 1935.
(205) MACBRYDE, C. M. J. Clin. Investigation **15**: 577, 1936.

DRUGS AFFECTING PARASYMPATHETIC NERVES

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The purpose of this review is to set forth the effects produced by the exhibition of a group of drugs which modify the action of parasympathetically innervated organs, in pharmacological as opposed to toxic concentrations. An examination of the older literature reveals the earlier experimenters were often, and naturally, at a loss for a criterion as to what constituted a reasonable dose range to be used in the analysis of the phenomenon under examination and the literature is confused by the citation of results of experiments which are of no physiological and only doubtful toxicological importance, as the more important toxic symptoms arise largely from effects on the central nervous system. One or two cases of this type are cited. Further, an indication is given of the development of our knowledge, and a discussion as to where and how the drugs may act.

Only literature that is necessary to illustrate these points is cited, but it is hoped that such citations will be adequate for any one wishing to pursue the matter more closely.

Atropine. The discovery of the inhibitory action of the vagus nerve on the heart by the Weber brothers in 1845 and of the secretory effect produced by the stimulation of the chorda tympani by Ludwig and by Claude Bernard in 1851, laid the foundation for the pharmacological study of the drugs to be considered in this review. The drugs are often termed parasympathomimetic drugs. The first known of the group does not mimic the action of nerve stimulation but rather the effect of abolition of the nervous action. This drug is atropine. The action of atropine was known from cases of poisoning by the berries or other parts of Atropa Belladonna long ere Mein in 1831 and Geiger and Hesse in 1832 isolated the alkaloid atropine. Indeed, according to Binz (1891), Johannes Weyer in the 16th century must have experimented with it for he describes the amusement caused if a person, before a good repast, were given a little vinous extract of the root and how the unfortunate victim was prevented by his dry mouth from sampling

the viands set before him. The fast heart was also known, but naturally more attention was paid to the toxic symptoms from the side of the central nervous system.

Bezold and Bloebaum in 1867 proved that in both the frog and the dog, vagus stimulation no longer produced a decrease in cardiac rate after atropine had been given. Schmiedeberg (1870) proved to his satisfaction that the effect must be produced on the endings of the vagus, since stimulation of the postganglionic fibres from the sinus crescent in the frog was also ineffective, while after nicotine such stimulation produced inhibition. Further it had long been recognized that atropine did not affect nerve fibres in their course through the tissues, and it was of course apparent that the heart muscle continued to carry on its functions.

This argument has not always been accepted, since Gaskel (1883) implies that the explanation might be that atropine brings the heart to such a state (of irritability or excitability) that vagus stimulation no longer is able to affect it. Nor to-day can this position be negatived or supported by the old observation going back to v. Schroff, Frohlich and Lichterfels (1852) that the heart rate is primarily reduced by small doses of atropine. This observation has been too frequently omitted from pharmacological texts, though frequently confirmed, more recently by Platz (1922), Rudolf and Bulmer (1924), McGuigan (1921). Though Cushny (1924 a) states that slowing may occur after cutting the vagi or after the vagus has been depressed by an initial dose, in these cases the dose causing slowing was undoubtedly high and toxic to the heart muscle. The difficulty lies in part in the fact that the early observers had no measure of what was to be considered a physiological dose. Kisch (1926) denies that a second small dose will decrease the rate in frogs. Rhode and Ogawa (1912) doubt the results of previous observers and their experiments showed that doses just adequate to prevent vagus inhibition did not change the oxygen consumption, while such doses as caused a secondary slowing did so.

Heinekamp (1922) seems to have shown clearly the slowing produced in dogs by doses as small as 0.3 mgm. of atropine in oil subcutaneously is due to an effect on the medulla, a central stimulation of the cardiac-inhibitor centre. Gremels (1936) has also shown that very small doses, 1 to 10 γ, to a cat may produce an increase in vagus irritability.

If the recent observations of Jourdan and Nowak (1936), which indicate that the vagus nerve contains fibres arising from the medulla whose stimulation produces cardiac acceleration, prove to be correct,

the acceleration that is sometimes observed after atropine with vagus stimulation in mammals, is amply explained.

The careful studies of Crawford (1924) and Marris (1916–17) reveal that while 1/100 grain will give only a decrease in rate of some 8 beats per minute, 1/50 to 1/33 will give an initial decrease followed by an increase of some 20 to 40 beats, the maximum rate being reached in some 20 to 30 minutes and the effect declining for an hour. Typhoid patients, even with the larger dose, gave a decrease, or at most an increase of 7 beats. The increase in normal persons was greatest in young adults, becoming much less over 70, and in some cases of disease, mitral stenosis, was still less. A maximum increase is only produced by 1/10 grain.

In 1872 Heidenhain showed clearly that the stimulation of the chorda tympani after atropine no longer led to salivary secretion, but stimulation of the sympathetic did so. He also proved that even after atropine, the blood flow through the gland increased on nerve stimulation. Langley's (1878 a) experiments indicate that in the dog 100 mgm. of atropine did not depress sympathetic stimulation, while only 15 mgm. depressed the chorda completely. Langley and Dickenson (1890) proved that while after nicotine chorda stimulation produced no secretory effect, stimulation by electrodes sunk into the neck of the gland still produced secretion, as postganglionic fibres were thus reached.

Alternating chorda with sympathetic stimulation, leads to the sympathetic producing a greater flow of saliva and MacIntosh and Rawlinson (1935) showed that this was true, even when after atropine chorda stimulation produced no secretion. Nor could this greater secretion be explained as due to a greater blood flow. While all this evidence does not prove that chorda and sympathetic stimulation act on the same cells in the gland, it does show that even after atropine has abolished the secretion produced by chorda stimulation, the action of this nerve produces changes which affect the cells reached by sympathetic impulses.

Barcroft (1901) reported that after atropine, chorda stimulation caused an increased CO_2 loss from the gland but that the uptake of oxygen which stimulation produced under normal conditions did not occur. Ewing (1911) has sh wn that atropine decreases the quantity of saliva in man and of its solids and amylolytic power, but the latter recovers first when secretion is not entirely suppressed.

Langley (1890) showed that the effect of stimulation of the nerve of

Jacobsen to the parotid gland is also depressed by atropine. Tear secretion is also depressed (Magaard, 1882).

Again, Riegel (1899) and Adlor (1900) produced evidence that gastric secretion was decreased in amount by atropine and that the secretion was less acid. Keeton, Luckhardt and Koch (1920) found that 1 mgm. completely inhibited gastric secretion in dogs, while smaller doses might decrease the quantity and the pepsin content more than the acidity. As in the case of the pancreas, differences in results in various experiments will depend on the presence or absence of hormonal secretion, as Babkin and Sawitsch (1908) showed that atropine did not affect the action of secretin. Polland (1930) showed that gastric secretion produced by histamine was decreased in total amount, acidity and pepsin content, while the hydrochloric acid percentage increased slightly. Uschakow's (1896) acute experiments on pithed dogs made clear that vagus stimulation after a long latency (minutes) led to gastric secretion and that atropine abolished this secretion. Uschakow, and apparently Babkin, hold that the long latency suggests that the vagus contains both augmentor and inhibitor secretory fibres.

Sawitsch and Ssoshentstwenskij (1922) demonstrated that the secretion of intestinal juice produced by vagus stimulation was decreased in quantity and ferment content by atropine.

Pawlow (1878) produced evidence that atropine decreased the pancreatic secretion in dogs and this was confirmed by Afanassiew and Pawlow (1878); but the irregular effects described by these and other workers are doubtless explained by the actions of hormones. As Babkin and Sawitsch (1908) and Bijlima (1911) showed, it did not abolish secretin juice while it could that caused by stimulation, as was clearly shown by Modrakowski (1906). The continuous secretion in rabbits could not be prevented by atropine (Pawlow, 1878; Gottlieb, 1894). A further difficulty was cleared away by Korovitsky (1923) when he showed that the vagus supplies contractile fibres to the ducts and that their contraction may decrease the flow. These also are depressed by atropine.

Cushny (1924b) states that Bernstein and Dogiel observed in 1866 that the contraction of the pupil produced by stimulation of the oculomotor was abolished by atropine, and Adamuck 1870 showed that this was true of stimulation of the postganglionic fibres, while the muscle still responded to stimulation. Anderson (1906) proved that while atropine removed or prevented the action of pilocarpine on the denerved sphincter, it did not prevent contraction when the

animal was asphyxiated. Poos (1927) working with the isolated sphincter or dilator muscle, demonstrated that atropine causes a fall of tone and decrease of movements in the sphincter pupillae, but had no effect on the dilator. The ciliary muscle is also depressed and these effects account in part, if not completely, for the increase in intraocular pressure.

That the vagus endings in the bronchial muscle are depressed was shown by Dreser (1889), Einthoven (1892), Beer (1892) and Weber (1914), by Pedden, Tainter and Cameron (1935) for the arecoline spasm, and Trendelenburg (1912) for the isolated musculature.

According to Szpilmann and Luchsinger (1881) the augmentation produced by vagus stimulation of the smooth but not the striated muscle of the esophagus, was depressed by atropine. Langley (1898), who repeated in dogs some observations of Openchowski (1889), found that the cardiac sphincter was supplied by both augmentor and inhibitory fibres from the vagus and that the inhibition became more marked after atropine in a dose of 10 to 20 mgm. A somewhat similar observation was made by May (1904), who found that vagus stimulation first produced a diminution of tone and movements of the stomach. Repeated vagus stimulation gave an increase in tonus, especially marked in the cardiac portion, while in the pyloric region increase in movements was more conspicuous. Atropine seemed to prolong the inhibition but even enormous doses, 100 mgm., did not prevent vagus stimulation from producing increased movements. While the inhibition might be explained did the nerves stimulated in these experiments contain sympathetic fibres, though this seems improbable, one cannot help being reminded of the evidence produced by Jourdan and Nowak (1936) that the vagus contains both augmentor and inhibitor cardiac fibres. Further, there is evidence that the state of gastric tonus influences the effect of drugs. Brown and McSwiney (1926) showed that a concentration of $1:1.25 \times 10^{-6}$ decreases the movements produced by pilocarpine in isolated gastric slips. There is, indeed, abundant and concordant agreement that the increase of tonus and gastric contractions produced by pilocarpine is readily decreased by small doses of atropine; and Titelbaum (1926) has shown that the gastric movements recorded by a swallowed balloon in man are decreased by atropine, even in a dose of 0.3 to 0.5 mgm., while larger doses, 1 mgm. and over, are necessary if food has been taken.

Small doses of atropine, 1 mgm. or less, often appear to leave the movement of the small intestine unaffected, though Bezold and Bloe-

baum (1867) described the gut as less active; and Harnack (1878) tested its effect in reducing the spasm of lead colic. Zunz and Tysebaert (1916) interpret their experiments as showing that atropine affects intestinal tonus more than movements, 0.001 mgm. per kgm. in dogs. Yet the importance of the effect of atropine has been obscured by many observers, who worked under such experimental conditions that pharmacological effects could not be demonstrated, but merely toxic ones. A typical example is the work of Magnus (1904) who had the merit of introducing the isolated gut method. His control of physiological bath conditions and technique was so poor that atropine appeared to produce no effect, save in a concentration of 1:4000 and in this concentration appeared to stimulate. His belief was strengthened by the experiments of Langley and Magnus (1905). Hence the common assertion in text books that small, or in the more discerning, large doses stimulate. Unger (1907) showed that atropine produced a decrease in tonus and movement in a concentration of 1:2 million and less. This Magnus (1908) denied as a result of repeating his work. There is no doubt on examining the tracings he adduces, that the bath fluid was responsible, probably too acid. All subsequent workers have confirmed Unger rather than Magnus, but even Cushny (1924c) accepts the results of Magnus rather than of the unimportant Unger.

Had Magnus regarded clinical observations, he would have been more doubtful of the value of his own. Doses believed by clinicians to affect intestinal spasms and which are certainly effective elsewhere in the body are 1 to 2 mgm. for a man of 70 kgm. (155 lbs.) one part in 70 millions body weight or 1:5 million in blood concentration. Toxic doses run from 10 to 60 mgm. so that a concentration of 1:½ to 1 million would be within toxic limits; 1:4000 seems fantastic.

There is do doubt that even low concentrations abolish the tonus increase produced by physostigmine and pilocarpine, as Unger first showed, and decreases the contractility of the gut in a concentration of 1:375 millions, Liljestrand (1919). Henderson (1922–23) produced evidence that doses of 0.1 to 0.2 mgm. in dogs decreased gut tonus. Plant and Miller (1926) showed in unanesthetized dogs that 0.2 mgm. decreased gut tonus and movements; and Ganter (1924) the same in human beings with 1 mgm. subcutaneously. Jacobi (1891), Liljestrand (1919) and LeHeux (1919 a) on better evidence than that of Magnus state that large doses may stimulate, but this does not seem to be clearly established, save perhaps when extremely high concentrations

are reached, which are of no importance. But Jacobi (1891), Bayliss and Starling (1899), Cushny (1910) and Henderson (1922) have all shown that the rhythmic movements of the gut are increased by vagus stimulation even after doses of 10 to 30 mgm. to a dog. Plexus-free small intestinal strips were first studied by Magnus (1905), but his technique throws doubt on the results. Gasser (1926) and van Esvald (1928) working with carefully controlled plexus-free preparations have shown that choline, pilocarpine and physostigmine may increase the tonus and the movements of plexus-free gut strips, and that atropine decreases this effect or decreases normal movements. The results are, however, somewhat irregular and the concentrations as a rule higher than for plexus-containing strips or normal gut.

Trendelenburg (1917) who suggested the method for recording peristalsis in the isolated guinea-pig gut, unfortunately used high concentrations of atropine and his results were irregular in some cases, causing gut activity, in other cases abolishing the intestinal response to rise of pressure, but if low concentrations are used there is a definite fall in gut tonus, but response occurs though a higher pressure is required to elicit them and the peristaltic response soon fails. There is as yet an unexplained relationship between gut tonus and peristaltic movements. The local stimulation of the contractile mechanisms is not abolished by therapeutic concentrations.

The movements of the intestinal villi are increased by vagus stimulation and the effect of such stimulation is abolished by atropine (v. Ludany and Jourdan, 1936).

The activity of the large gut in man is decreased by 1 mgm. of atropine, Ganter and Stattmuller (1924), Katsch (1914). Langley and Anderson (1895) showed that even after large doses, pelvic stimulation caused contraction of the colon. The urinary bladder suffers a fall of tonus with 1 mgm. or less of atropine, while even after large doses up to 100 mgm. in cats contraction of the same extent is produced by sacral root stimulation (Langley, 1912) (Henderson, 1922). Yet the character of the contraction changes, Henderson and Roepke (1934–35). Before the administration of atropine, a urinary bladder contracting against an opposing elastic resistance produces a flat-topped curve which lasts slightly longer than the stimulation and declines slowly. After atropine, while the pressure produced is as great, the fall occurs abruptly even during stimulation. It was for this reason that Henderson and Roepke postulated two mechanisms, a contractile one not depressed by atropine and a tonus mechanism

readily depressed. It is by no means impossible that the intestinal canal possesses two such mechanisms, certainly contraction occurs on vagus stimulation after atropine, but tonus remains low.

On sympathetic innervations. Luchsinger (1877) observed that the sweat secretion of the pads in cats was abolished by atropine and Langley and Anderson (1891) proved that the sweat-producing nerve fibres were sympathetic in origin.

Vessels. While there is abundant evidence (Cushny 1924 d) that large doses or high concentration of atropine dilate vessels, this falls without the range of our discussion, though occasionally in susceptible persons a reddening of the face occurs with pharmacopoeial doses. Yet in certain vascular areas where sympathetic stimulation causes vasodilatation due to acetylcholine production, atropine annuls this effect (Dale, 1914; Hunt, 1918; Bulbring and Burn, 1935).

In the isolated guinea pig uterus, pilocarpine causes a contraction and atropine abolishes this effect (Führer, 1916; Cushny, 1910; Fardon, 1908).

It is evident that atropine diminishes or in higher concentrations prevents the normal effect of nerve stimulation in the case of all examined parasympathetic and many sympathetic endings, but the concentration necessary to completely prevent or to diminish the effect of stimulation varies with the organ in question as Henderson (1923) has shown and is clinically well known. A dose of $\frac{3}{4}$ mgm. (1/100 gr.) will decrease salivary, bronchial and nasal secretion and diminish gut tonus; yet the heart rate is decreased, not increased. While in a dog 1 mgm. will usually completely abolish the effect of cardiac vagus stimulation, it requires 5–15 mgm. to completely remove the effect of chorda stimulation. The facts cited in regard to typhoid fever again indicate that the functional state must be considered (Marris, 1916).

Hyoscyamine. Pure laevo-hyoscyamine seems to have been first obtained by Gadamer. Most of the early work, and indeed much of the later, was carried out with impure preparations. Cushny (1903) however, using a pure laevo form, was able to show that it was twice as active as atropine, its racaemic form, in its action on the pupil, the cardiac vagus and saliva. Dextrohyoscyamine was only 1/12 to 1/15 as active as the laevo form in these cases. These observations have been substantially confirmed by von Oettingen and Marshall (1934), though in the case of the rabbit, owing to the destruction of the alkaloids by the serum, the ratio does not hold; and by Jendrassik and Will (1930) in good experiments where low concentrations were used, who

showed that the ratio of laevo-hyoscyamine to atropine held for the isolated gut. Laidlaw (1909) seems to have found that the laevo form was 100 times as active as the dextro on the iris and 25 times on the vagus. Though the hyoscyamines have not been studied on all the organs, there is reason to believe that the effect of laevo-hyoscyamine will be precisely that of atropine. It may be noted that Pilcher (1934) found that in children the blush caused by atropine was also produced by laevo-hyoscyamine, and in the same ratio as Cushny established. He attributes this to a central effect, but it is quite possible that it too is peripheral in origin.

Hyoscine. The early papers on this alkaloid, which are well reviewed by Kochmann (1903), were nearly all carried out with impure specimens of the alkaloid, and indeed Cushny and Peebles (1905) seem to be the only authors who adequately studied pure preparations. They found that on the salivary secretion the laevo form acts twice as strongly as the racaemic, and also on the cardiac vagus. The laevo form appears to be roughly 5 times more active than laevo-hyoscyamine; and the other authors who have studied its action seem to agree that it is more potent, but its effect disappears more rapidly.

Homatropine. This was isolated by Ladenburg and was studied by Bertheau in 1880. He found that it led to an increase in cardiac rate in dogs and in rabbits and prevented the effect of vagus stimulation. It caused dilatation of the pupil and removed the effects of muscarine. Tweedy and Ringer (1880) also observed its effect on the heart, pupil and sweat glands. The pupil is more rapidly dilated than by atropine, but the effect disappears more rapidly. This has been confirmed by many observers. Jendrassik and Will (1930) showed that homatropine was about 1/10 as effective as atropine in removing the pilocarpine effect from gut.

Muscarine. Although certain of the effects produced by muscarine had long been known through cases of poisoning by mushrooms, it was not till Schmiedeberg isolated the alkaloid from Amanita muscaria that its effects could be accurately studied, as mushrooms also contain choline. Schmiedeberg and Koppe (1869) demonstrated the decrease in cardiac rate, the salivation, the temporary constriction of the pupil, the increased intestinal activity, the dilatation of vessels of the rabbit's ear and the expulsion of urine and faeces. There was at first much difference of opinion as to how these effects, particularly the decrease in cardiac contractility and rate, were produced. It might be due to a poisoning of the muscle or a stimulation of the vagus inhibitor mecha-

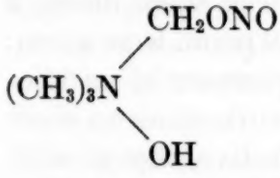
nism. Observations such as those of Löwit (1882) led to confusion, as he was unaware that the vagus in frogs contained sympathetic fibres. Rhodius and Straub (1905) doubt certain of the previous observations; they found that a second dose never increased the effect of the first. These workers and Cushny (1893) described clearly cases with decreased rate, contractility being unaffected or apparently increased, owing to increased filling time; or decrease in both rate and contractility and decrease in conductivity; auriculo-ventricular block, while in other cases the ventricle appeared to cease contracting while both auricle and sinus were active; loss of auricular contraction without loss of conduction and decrease in tonus. Gaskell (1882) described experiments in which the effect of muscarine could be increased by vagus stimulation, though this is denied by Jonescu (1909) who found that muscarine even when the heart rate was not reduced, had decreased the effectiveness of vagus stimulation. The numerous experiments by Kobert, (1886), Harnack and Witkowski (1876) and those above mentioned and others, finally led to the belief that muscarine stimulated the inhibitory vagus endings. Rothberger and Winterberg (1910) showed that the vagus in dogs failed to produce its usual effect after muscarine. Ott and Field (1878) proved that the sweating produced by muscarine was due to a peripheral action. Prevost (1874) confirmed most of the observations of Schmiedeberg and demonstrated an increase in intestinal and pancreatic juice and bile. Dixon and Brodie (1903), Trendelenburg (1912) (on isolated muscle) and Weber (1914) have all shown that muscarine produces a constriction of the bronchi. Battelli (1897) demonstrated the increase in gastric activity produced by muscarine with the balloon method, and Schütz (1886) with isolated dogs' stomachs.

The knowledge of the action of muscarine on the intestine was extended by Bayliss and Starling (1899), who unlike the earlier workers using merely visual observations, recorded the effects with the balloon method and found that 0.3 mgm. to a dog intravenously produced an increase in tonus and rhythmic movements. Gasser (1926) and van Eswald (1928) showed that muscarine caused a stimulation, but according to the latter plexus-containing strips were some 100 to 200 times more sensitive than plexus-free.

Hanc (1898) showed that the body of the urinary bladder contracted with muscarine, while the sphincter relaxed.

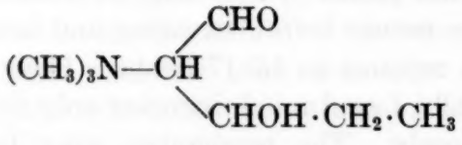
The whole picture of the action of muscarine has been somewhat confused by the discovery by Schmiedeberg and Harnack (1877) of

an artificial muscarine produced by the action of nitric acid on choline. Dale (1914), aided by Ewins, has clearly shown that this synthetic, pseudo, nitric acid muscarine is in reality a nitroso-choline



. Dale also describes a nitric derivative. The synthetic muscarine resembles in its effects the natural product, but differs from it both qualitatively and quantitatively. The action on the pupil is much less, it produces less vasodilatation and as Boehm (1885) showed, has a curara-like action on skeletal muscles.

The recent work of Kögl, Duisberg and Erxleben (1931) has shown quite clearly that natural muscarine is a choline derivative with a branched side chain containing a terminal aldehyde group.



Pilocarpine. The extract of pilocarpus was first used, according to Binz (1891), by a Brazilian physician Cutinho (1874) who showed its ability to produce marked sweating; and various extracts were promptly studied on patients, which disclosed its gross effects. The alkaloid pilocarpine was isolated in 1875 by Gerrard and by Hardy, while Weber (1876) published the first observations on the pupil and sweat and salivary glands. Erich Harnack and Meyer (1880) made the first detailed study of its action. They showed that pilocarpine caused either an arrest or a decrease in cardiac rate in frogs and mammals and that vagus stimulation, when the heart was slow, caused merely an acceleration; and also in mammals vagus stimulation failed to have its usual effect after pilocarpine. The pupil was contracted by local application, the gut showed increased activity and spasm, as did the uterus and urinary bladder. It also caused bronchial secretion. All these effects were abolished or prevented by atropine. MacLean (1908) made a closer study of its action on the frog's heart. He found that pilocarpine and muscarine only produce inhibition if stimulation of the crescent were effective and if the crescent were repeatedly stimulated, then neither pilocarpine nor muscarine produced inhibition. When pilocarpine was effective, tonus, rate, contractility and conduc-

tivity were all decreased. Gaisbock (1911) showed again that pilocarpine decreased the effect of sinus stimulation. Loewi (1912) found that this decrease in effectiveness of vagus stimulation occurred regularly in frogs. In rabbits too, it occurred with even small doses, 2 mgm., at times, but in other cases did not even if 89 mgm. were given; nor does he consider the failure as due to the occurrence of an idioventricular rhythm. He quotes experiments and authorities to show that even vagus stimulation itself may become ineffective, though with smaller doses there is a summation of the effect of vagus and drug. Marshall (1904) had found that pilocarpine, 10 mgm. to a rabbit, was ineffective after prolonged vagus stimulation. Repeated doses of pilocarpine also lose their effect.

Curschmann (1877) first called attention to the fact that with therapeutic doses of pilocarpine in man, the cardiac rate is increased by a few beats per minute and a decrease does not occur. Various observers have dealt with this point. According to Blomberg and Ronnell (1930), the increase occurs before sweating and lasts several minutes, up to 30, and may amount to 13–17 beats. Their normal rates were low and Platz (1922b) found much increase only in vagotonic persons who sweated profusely. The mechanism may be reflex from the activated glands, or in some cases is probably due to the liberation of epinephrine.

Langley (1878b) noted that pilocarpine produced saliva, and his experiments suggest that pilocarpine in certain doses decreased the effectiveness of chorda stimulation. Pilocarpine acts even after the chorda has been previously cut for days (Maevsky, 1923), though it appears less effective (Fleming and MacIntosh, 1935), probably because the gland has decreased in size.

There is a great difference of opinion as to the character of the saliva produced, even from the submaxillary gland alone. Langley, for example, says that it is more viscid in dogs. This probably depends on factors as yet unrevealed (Lucas and Henderson, 1936). Ewing (1911) showed that 10 mgm. produced a long-continued ($1\frac{3}{4}$ hour) flow in man, which might amount to some 350 cc., with increasing and then decreasing content of solids. The total amount of ptyalin is increased, but not necessarily its percentage. The secretion of tears, nasal mucus and bronchial secretion (Leyden, 1877) is also increased by pilocarpine.

The effect of pilocarpine on pancreatic secretion is not great and might be explained as due to the contraction of the ducts (Korovitsky,

1923). Gottlieb (1894) found that it produced an increase in the continuous secretion in rabbits, but his experiments are not free from the possibility of the increase being due to secretin, as the increased secretion reported by Camus and Gley (1913) appears to be, since they removed the whole of the small intestine.

The early experiments of Riegel (1899) which appeared to show an increase in gastric secretion with pilocarpine, needed confirmation in view of the possibility of an hormonal effect. Zitowitsch (1902) showed that the amount of gastric secretion was much less in amount and was more tardy than that of the salivary glands and needed larger doses to produce it. Amantea (1924) showed that 4 to 10 mgm. caused a secretion almost as acid as that produced by feeding meat or bread. Toby (1936) showed that pilocarpine produced a gastric secretion resembling that produced by feeding, as it was rich in pepsin, contained more mucin and a lower acid content than that produced by histamine.

That the intestinal secretion was increased, was shown by Vella (1881) and others and clearly confirmed by Schepowalnikow and by Sawitsch (1899), and atropine abolished this action. Luchsinger (1877) showed that the secretion of sweat was produced by a peripheral action.

The original observations that pilocarpine applied locally led to a contraction of the sphincter of the pupil, have been confirmed by many workers, but to Anderson (1906) goes the credit for the most detailed examination of the phenomenon. He found that pilocarpine was even more effective than normally, after cutting the preganglionic long ciliary nerves and after removal of the ciliary ganglion. It also caused pupillary constriction after the postganglionic fibres have degenerated; in each condition the effect of pilocarpine was removed by atropine (also Niitani, 1929). The ciliary body also contracts. Poos (1927) confirmed the effect of pilocarpine on the isolated sphincter muscle and showed that it had no effect on the radial one.

Winkelstein and Aschner (1924) proved that pilocarpine produced a contraction of the gall bladder and also increased the tonus of the sphincter of Oddi. Various observers have shown that the increased tonus produced by pilocarpine is diminished by atropine (Ivy, 1934). That the bronchial musculature is contracted by pilocarpine was shown clearly by Dixon and Brodie (1903) and Weber (1914), and on isolated muscle by Trendelenburg (1912). The concentration or dose required to produce much effect is above that to produce salivation. The increased activity and tonus of the gastric musculature was shown

by Schütz (1886), Batelli (1897) and Ganter (1924), who found that 10 mgm. in man increased both tonus and contractility, as judged by the swallowed balloon method.

That pilocarpine produces a rise of tonus and increased rhythmic movements of the intestine, has been shown by many observers, for example Bayliss and Starling (1899) with the balloon method. Neukirch (1912) showed in isolated rabbit gut a tonus increase in a concentration of 1:10–1:20 millions pilocarpine, and increased contractility. Gasser (1926) and van Eswald (1928) showed its action on plexus-free strips.

Trendelenburg (1912) showed that a concentration which did not appear to produce any effect on the isolated guinea pig gut, led to the gut showing a peristaltic response to a lower pressure. Trendelenburg's tracings suggest a slight increase in tonus and this has certainly occurred in all cases in the authors' experience when the threshold for pressure was decreased. Bergman and Katsch (1913) and Ganter and Stattmüller (1924) have made evident the increase in tonus and activity produced by pilocarpine in therapeutic doses in man.

The urinary bladder contracts on the administration of pilocarpine, as do the ureters, in spite of their purely sympathetic innervation.

Fühner (1916) observed that the isolated uterus contracted when exposed to a concentration of 1:1 million, though Fardon (1908) and Kehrer (1907) had found much higher concentrations necessary and Cushny (1910) found that it caused contraction of the pregnant uterus of the cat and relaxation of the non-pregnant, in both cases paralleling the action of the hypogastric. The pilocarpine action was promptly abolished by atropine, but not the effect of nerve stimulation. Unfortunately, he does not state the doses used and probably his results are due to ganglionic stimulation or adrenaline secretion, as explained by Dale and Laidlaw (1912).

Dale and Laidlaw showed that pilocarpine led to a secretion of adrenaline and caused a stimulation of ganglia in doses of about 4 mgm. to a cat.

The experiments of Brodie and Dixon (1904) showing that pilocarpine produced vasodilatation are of little value, but those of Hunt clearly prove its dilating action on certain vessels. The vessels of the submaxillary glands are dilated by doses just causing secretion, as are those of the genital organs.

Arecoline. Arecoline was isolated by Jahns in 1888. Marmé (1890) seems to have been the first to have made a study of the pure alkaloid.

He noted that it constricted the pupils promptly, decreased the rate of frogs' hearts, caused bronchial and salivary secretion and increased peristalsis. Lavagna (1895) showed that on local application to the conjuctiva, it has a more marked effect on the pupil than pilocarpine, but somewhat less than physostigmine. The pupillary action occurred quicker than with pilocarpine and the ciliary body was less affected than the iris. Föhner (1894) showed that it acted on the intestines like pilocarpine, caused contraction of the spleen and bronchi. Meier (1907) showed that it decreased the activity of the frog's heart in very low concentrations, even after atropine. Pätz (1910) studied particularly its effect on the intestinal canal, where it increased movements in the intact animal. On the isolated gut it caused increase in the rhythmic movements in a concentration of 1:80 millions or less. These concentrations caused little or no increase in tonus, but 1 in about 20 millions produced a maximum tonus increase. Trendelenburg (1912) showed that it acted on isolated bronchi and was more active than pilocarpine, though there are no exact figures. The evidence shows that arecoline causes increased activity of all the organs supplied by parasympathetic fibres which have been tested, and acts on smooth muscle in a similar fashion to pilocarpine, but in all cases where exact comparisons have been made or may be drawn from the valid experiments, it seems to be more active than pilocarpine, and save for the doubtful experiments of Busquet (1933), no qualitative difference in action from pilocarpine.

Physostigmine. Calabar beans were brought to England in 1840 and Christison (1855) and Fraser (1863) studied the effects of their extracts, as did many observers in Europe. Erich Harnack and Witkowski (1876) first studied the effects of the pure alkaloid and showed that some of the effects produced by the extracts were due to other alkaloidal principles.

Harnack and Witkowski showed that the application of 0.5 mgm. in solution to an exposed frog's heart caused a decrease in rate, with larger beats. During this stage stimulation of the vagus or the sinus no longer produced cardiac arrest, but only a decrease in rate. If the frog's heart action had been arrested by muscarine, physostigmine locally applied produced beats, but not a restitution to normal, as atropine did. They then perfused the frog's heart and obtained, dependent in part on the strength of the solution used, decrease in rate with increase in tonus and arrest in a partial systole, a condition not relieved by atropine; or a slower rate and finally an arrest in diastole.

Further perfusion with 1:5000 solution for a short time made a previously active vagus inactive. In all their experiments the dosage applied must have been high. Yet it is apparent that the most marked effect with smaller dosage or in the early stages of large ones, is a decrease in rate. This might be due to a stimulation of the vagus and was abolished by atropine. The increase in tonus and other effects of higher dosage appears to be due to an action on the muscle.

Hedbom (1898), working with isolated hearts (rabbits, cats), showed that strong solutions caused a great rise in tonus, but that 1:100,000 caused only slowing and decrease in excursion. Solutions 1:5000 caused increase in tonus after atropine. Kobert (1886) also performed experiments which indicate a direct stimulating effect of physostigmine, for example a frog's ventricle at rest after a second Stannius ligature can be made to beat by the application of a physostigmine solution. Here again no real evidence is found by which one may judge of the absorbed amount and consequently whether the dosage was small or large. A dose of 2 mgm. intravenously to a cat caused a little decrease in cardiac rate but no effect on pressure, while 5 mgm. produced a primary rise in pressure (probably vascular) and then a decrease in rate with some decrease in cardiac tonus, these effects are promptly abolished by atropine. Further injections of physostigmine produce less and less effect and vagus stimulation becomes ineffective.

Various observers have, however, called attention to a much more important phenomenon, namely, that after a small dose of physostigmine which produces no apparent cardiac effect, vagus stimulation becomes more effective, i.e., either the same amount of slowing is produced with less voltage, or a greater or longer slowing with the same stimulus, Winterberg (1907) 0.1 mgm. or less per cat, Loewi and Mansfeld (1910). The latter also showed a similar increase in irritability for the chorda and Dixon and Ransom (1924) for the vagus to the bronchial musculature, while Anderson (1906) describes a similar effect on the ciliary nerve.

As Tsehboksaroff (1911) and Stewart and Rogoff (1921) have shown that the injection of physostigmine leads to a release of adrenaline, probably due to ganglionic stimulation, this factor must be considered when dealing with the effect of physostigmine, both on the heart and circulation.

The earlier observers working with calabar extract had discovered the contraction of the pupils and this was confirmed by Harnack and Witkowski (1876) for the alkaloid, and since that time it has had a

wide clinical use. Anderson (1906) showed that while it acted after section of the postganglionic ciliary nerves, it failed to produce its effect after they had degenerated; confirmed by Niitani (1929). Poos (1927) showed that it contracted an isolated sphincter but had no effect on the radial fibres.

Physostigmine causes contraction and increased peristalsis in the stomach and small and large intestines, Harnack and Witkowski; and Schütz (1886) confirmed this in the isolated stomach. Battelli (1897) confirmed this action for the stomach in man, Subbotin (1869), Jacobi (1891) for the intestine in animals, Unger (1907) on isolated gut strips; and Gasser and van Eswald showed that occasional plexus-free strips might react. Plexus-containing strips were more sensitive (1:100, 000 to 1:15 million). According to Dixon and Ransom (p. 806) small doses 2 mgm. for a cat and 4 to 5 for a dog, increase the irritability of the vagus to the gut.

The bronchial muscles are constricted and again the effect of vagus stimulation is increased (Dixon and Brodie, 1903). Trendelenburg (1912) showed that it constricted isolated bronchial muscle. Again Dixon and Ransom state that 1 mgm. physostigmine brings about an increase in the tonus and rhythmic movements of the urinary bladder of cats and dogs, which are diminished by 0.5 mgm. of atropine, but they state the tonus increases again. Kehrer (1907) also, with isolated uterus, found increase of tonus and movements with 1:200,000. As Subbotin pointed out, physostigmine also contracts the ureters. Physostigmine also causes increased salivary secretion even after the ganglia are paralysed with nicotine, and as Heidenhain observed, it increased the effectiveness of chorda stimulation after atropine. All the other glands of this group, lachrymal, parotid and bronchial, are also stimulated by physostigmine. The secretion of the pancreas in rabbits is slightly increased and also its solid content (Gottlieb, 1894). Pewsner (1906) produced evidence that the gastric secretion is increased by doses of 0.2 to 0.3 mgm. per kgm. in dogs. It also produces a secretion of sweat.

Nicotine. Posselt and Reimann isolated the liquid alkaloid nicotine in 1825. A case of poisoning directed attention to its action in 1851 and led to experimentation on animals by Orfila (1843), Bernard and van Praag (1855) and others. Hirschmann (1863) performed experiments which showed that nicotine depressed the cardiac vagus and the cervical sympathetic so that stimulation no longer decreased cardiac rate or dilated the pupil. Mayer and Truhart showed that stimulation

of the crescent in frogs was effective when nicotine had abolished the effect of vagus stimulation. Heidenhain (1872) described the effect of nicotine on submaxillary secretion. Langley and Dickenson (1889) showed that the local application of nicotine to the superior cervical ganglia led first to its stimulation and then to its depression and in a further series of researches (Langley 1890–1901) this observation was extended to other accessible ganglia with the same result. Hence the effect of intravenous administration can be clearly interpreted and it became evident that nicotine first led to a stimulation at the ganglia and then a depression, such that a stimulation of the pre-ganglionic fibres was ineffective. It was also clearly shown that the stimulation with any but the smallest effective doses was temporary and passed into a depression, while with larger doses the stimulation phase might not be evident. The work of Feldberg and Vartiainen and of Eccles has clearly shown that the effect of nicotine is on the ganglionic cell, and that when any effect is produced by preganglionic stimulation, acetylcholine is still produced. Langley (1901) had demonstrated that nicotine acts on sympathetic ganglia after the degeneration of the pre-ganglionic fibre and must then react with the cell.

Choline. According to Boehm (1885) Gaehtgens seems to have been the first to describe the action of choline and Boehm's work served to confirm and extend that of his predecessor. When injected, choline produced a flow of tears, salivation, temporary constriction of the pupil and slight decrease in rate of the heart of the frog, while Gaehtgens had obtained complete arrest, but in cats with 10 to 20 mgm. a rise in blood pressure with a slight decrease in rate. Müller (1910) confirmed this decrease in rate and showed that it occurred even if the vagi had been cut. He also showed that it caused a dilatation of vessels of the limbs, gut, kidney and brain, a rise of tonus even in supposedly plexus-free gut strips and a contraction of the uterus and iris. A short flow of saliva was obtained from the submaxillary gland of the cat with 5 mgm. and an increase in blood flow through it. Atropine abolished these effects and converted the fall of blood pressure into a rise. This was probably due to a stimulation of the sympathetic ganglia, as was shown by Dale's (1914) careful study, where 1 to 10 mgm. intravenously to cats caused a fall in pressure due to a peripheral vasodilatation, while after atropine 25 mgm. caused a rise. Atropine prevented the peripheral vasodilatation but did not prevent the stimulation of ganglia by choline.

Handowsky and Pick (1912) showed that choline produced some dilatation of the vessels of the frog. Chang (1929) presented some evidence that choline caused gastric secretion from a Heidenhain pouch.

The whole subject of the action of choline was lifted into another realm by the work of Hunt and Taveau (1906), though some years elapsed before the full fruits of this work became evident. But from the publication of the fuller work in 1911 the question of the action of choline became in most respects subordinate to that of its derivatives.

The first striking effect of Hunt's discovery was the use of the method of acetylation for the detection of choline. LeHeux (1919b) found that the fluid in which active strips of gut had been immersed contained a principle which increased the activity of other gut strips and he was able to show chemically that the principle was choline, whose action could easily be identified by the extraordinary increase in its activity by acetylation. Further, when the gut had been washed repeatedly it was inactive and the activity could be restored by choline. This led him and Magnus (1920) to consider choline as the hormone for gut activity. Indeed, a paper by LeHeux (1921) suggests that the needed principle was acetylcholine, as the addition of sodium acetate to the washed gut restored its activity. In 1914 Dale published an important paper on the action of choline esters, which has in part been referred to above. He completely confirmed Hunt's account of the activity of acetylcholine, as little as 0.001 γ (one millionth of a milligram) causing a recognizable fall in blood pressure. It took 10 γ to cause a slight cardiac slowing and this did not occur after atropine. If the dose of acetylcholine were increased, particularly after atropine had been given, a rise of blood pressure occurred, which Dale considered was due to a stimulation of sympathetic ganglia as it was abolished by nicotine and was not central in origin.

Owing to the review published by Alles (1934) and that by Gaddum and Dale (1936) and the fact that another review of the whole development of the action of acetylcholine and its release by various nerve endings is to be published in these REVIEWS, it appears unnecessary to discuss the discovery of the humoral transferal of the nerve impulse by acetylcholine by Otto Loewi (1921–24) which has had such a profound influence upon physiological and pharmacological thought. Much of this work, however, is fundamental to any consideration of the site of action of the drugs we are reviewing, and will be adduced in this connection. Hence only a few points essential to our further consideration will be referred to here. Loewi and Navratil (1924) showed that acetylcholine was produced in the heart by stimulating the vagus nerve, even when atropine had prevented any apparent effect of the stimulation. Gibbs and Szelöczey (1932) and Henderson

and Roepke (1932–33) showed that this was true in the case of the stimulation of the chorda tympani, and has also proved to be the case in other examples, e.g., Dale and Feldberg (1934) in the case of the stomach. Further, Loewi showed that physostigmine prevented the action of the choline esterase and accounted for the potentiation of the effect of stimulation. The work of Kibjakow (1933) as extended by Feldberg and Gaddum (1934), shows that acetylcholine serves as the means of transferal of the impulse from the preganglionic fibres of the superior cervical sympathetic to the ganglion cell and leaves little doubt that in this case the acetylcholine is produced by the nerve endings and not by the cell (Feldberg and Vartiainen, 1934).

These discoveries led Dale (1933) to suggest the descriptive term cholinergic for those nerves whose stimulation leads to the peripheral release of acetylcholine. Unfortunately this term is not synonymous with parasympathetic and the suggestion of Frank, Nothmann and Hirsch-Kaufmann (1923) that those structures responding to acetylcholine should be considered as parasympathetic no longer holds good, and the older idea of Hans Meyer that the purely anatomical division of the autonomic nerves into sympathetic and parasympathetic is pharmacologically meaningless, receives support, at all events as far as concerns the characteristics of the post-ganglionic fibres. Preganglionic fibres, even of the sympathetic, are cholinergic and the work of Henderson and Roepke (1934–35) as well as that of others, certainly suggests that the parasympathetic preganglionic fibres are of the same type. Dale, Feldberg and Vogt (1936) and Brown, Dale and Feldberg (1936) have presented evidence that acetylcholine also serves as the transmitting agent of motor nerve impulses to voluntary muscles.

Site of action. The older conception that the site of action of these drugs was on nerve endings, was founded upon the parallelism of their effects on smooth muscle or glands cells with electrical stimulation of the appropriate nerves, and was well summed up by Dixon and Ransom (1912). Pilocarpine, which had been more thoroughly studied than muscarine or choline, everywhere produced effects that were very like those of nerve stimulation. Doubt has alone been thrown on the identity of the effects of nerve stimulation and pilocarpine action in the case of the salivary secretion (Babkin, 1931). Atropine abolished the effects produced by pilocarpine, muscarine and choline and of nerve stimulation, save in the case of the gut and urinary bladder. Attention may here be called to the fact that the effects of nerve stimulation are

not the same after atropine as before it in the case of the urinary bladder, and that subsequent study may reveal the cause of this apparent, but possibly not real, exception. In regard to physostigmine, a certain difference had been noted, namely, that it potentiated nerve stimulation within certain limits. As Dixon and Ransom quite correctly point out, the experiments of Anderson on the iris sphincter, which showed that after degeneration of the postganglionic fibres, physostigmine no longer acted, forced a modification of the prevailing opinion and led to the view that while the nerve ending degenerated, a non-degenerating myoneural junction or receptive substance persisted on which pilocarpine, muscarine and atropine acted.

The reason why this hypothetical myoneural junction was invented lies partly in the same anatomical conceptions as had led to the nerve ending being selected as the site of action, and secondly, by the conception that a cell having been depressed (or as it was often put, paralysed) by a drug, would be unable to react to other chemical stimuli. Anderson had shown that the deafferented iris sphincter contracted on asphyxiation, even after atropine in quantities adequate to remove the action of pilocarpine had been applied. The anatomical cell could not have been "paralysed" by atropine if it reacted to carbon dioxide. Regarded chemically, this conception seems gratuitous.

The work initiated by Loewi on the transferal of the nerve impulses to the cell by acetylcholine, at once suggested new fields of thought. Where is the acetylcholine produced, at the anatomical nerve ending, the muscle sole, the cell? While this question cannot be answered with certainty, the evidence presented by Feldberg and Vartiainen (1934) and Eccles (1935) seems to show that it is produced by the preganglionic nerve endings in the case of the superior cervical sympathetic ganglion, as antidromic postganglionic stimulation does not lead to its liberation, and as it is still produced when the postganglionic cell fails to transmit an impulse owing to the presence of nicotine and acetylcholine or eserine in excess. We do not know its precursor, but that bound acetylcholine must exist, is evident from the fact that from an organ with the least destructive technique that has as yet been devised, more acetylcholine can be obtained than suffices to produce maximum physiological activity (Chang and Gaddum, 1933).

That the acetylcholine acts on the cell seems proved; and that it sets up a series of chemical reactions is probable, first from the analogy with skeletal muscle, where a series of chemical reactions occurs with or preceding the contraction, and from Feldberg and Guimarais'

(1935) analysis of Secker's erroneously interpreted observation on the salivary gland. These chemical processes must be cellular. Further, as Cattell and Wolff (1935) have shown, acetylcholine stimulates the denervated sphincter iridis. Rosenblueth and Cannon (1934) have adduced evidence that the denervated smooth muscle cell cannot be stimulated electrically, but only chemically, and this has been in part confirmed by Bacq and Monier (1935). It is of course still possible to suggest that the reaction occurs through the hypothetical myoneural junction, but this seems entirely unjustified. We may then more simply think of acetylcholine, set free by the nervous impulses or applied, acting upon the cell, producing certain chemical and physical results. Using the old anatomical conceptions, atropine must act more peripherally. This phraseology now seems ridiculous. Atropine must react with the cell. Further, it is evident that the reaction of atropine with the cell does not prevent the initiation of the physicochemical disturbances bringing about the liberation of acetylcholine, probably from the nerve ending itself. The reaction of the cell with atropine has merely prevented either the attachment of acetylcholine to the cell or its response to the acetylcholine attachment. That the latter is more probably the case may be inferred from the facts adduced by Hunt (1915) and Hunt and Renshaw (1934), who showed that the action of acetyl-β-methyl choline on the blood pressure and heart was only partially removed by a dose of atropine adequate to remove the effects of certain other choline derivatives. A subsequent dose of the choline produced effects, though less than before the atropine. Both the acetyl-β-methyl choline and the atropine were apparently acting on the cell.

Muscarine as a choline derivative may then be thought of as reacting with the cell. Pilocarpine it seems must react with the cell. It certainly appears to prevent the effect of nerve stimulation upon the cells in concentrations which produce stimulation, as mentioned above. The mutual antagonism between pilocarpine and atropine cannot be explained by any chemical reaction between these alkaloids and there seems every reason to suppose either that atropine displaces pilocarpine from the cell and vice versa, or that the effect produced upon the cell by its union with pilocarpine is prevented by atropine. There seem as many pharmacological reasons for believing that pilocarpine reacts with the cell as for muscarine, for example, both decrease the effect of nerve stimulation and muscarine, a choline derivative, undoubtedly reacts with the cell.

Physostigmine, according to the evidence of Anderson, does not react with the denervated cell. That the cell, after the loss of nerve impulses, is not the same is evident from its increased sensitivity to pilocarpine. Whether chemical changes occur in the smooth muscle cell after denervation, similar to those that occur in skeletal muscle, we do not know; but it may be noted that after motor nerve degeneration the skeletal muscle cell becomes more sensitive to acetylcholine. The work of Langley (1906) and Edmunds and Roth (1908) seems to show that the effects of physostigmine on skeletal muscle after nerve degeneration can be removed only in part by atropine. It appears then that physostigmine in concentrations which may be considered physiological reacts with certain skeletal muscle cells. This apparent reaction with the cell might possibly be explained as an action on the esterase, if in degenerating muscle there were a continuous liberation of acetylcholine, such as Henderson and Roepke (1933) found occurred on prolonged perfusion of the submaxillary gland. The work of Harnack and Witkowski and of Hedbom certainly suggests that in high concentrations physostigmine reacts with the heart muscle cell, but that it does so in physiological concentrations is not proven; though as it reacts with the enzyme, and this is cellular in part at least, it is probable that it does so. Feldberg and Vartiainen (1934) also have presented pretty conclusive evidence that eserine reacts with the ganglionic cell, both in low concentrations up to $1:10^6$, when it appears to sensitize the cell, and in high concentrations when it causes a depression, though acetylcholine is still produced on stimulation. Its important pharmacological effects seem to be adequately explained by its depression of the esterase, so that any acetylcholine produced by nerve impulse may act for a longer time or in higher concentration without destruction. The esterase in question may be in part a cell constituent, in part free in blood or lymph. The adherents of the myoneural junction hypothesis may attempt to gain support from the fact that physostigmine causes muscular or glandular activity after section of pre- and even postganglionic fibres, when it might be assumed that no acetylcholine was being liberated. Yet the precursor of acetylcholine is very labile and under the abnormal conditions present might escape; and further, many facts have been adduced which point to an action of physostigmine on cells. If Engelhart's (1931) experiments are to be trusted, the presence of acetylcholine and probably its precursor disappears from the iris and ciliary body after degeneration of the postganglionic oculomotor fibres.

There is, however, other evidence that conflicts in part with the above view that the site of action of acetylcholine, muscarine and pilocarpine is on the cell and not on the nerve ending. It has long been known that muscarine and pilocarpine act on the auricles of turtles, but not on the ventricles. This is also true of acetylcholine, to which the auricles respond in what may be considered physiological concentrations, while the ventricles are affected only by ones far above the physiological. The auricles are reached by the vagus; the ventricles are not. The heart of the chicken embryo in its early stages before an anatomical connection has been made with the vagus, fails to react with physiological concentrations (Cullis and Lucas, 1936; Markowitz, 1931; effective concentrations 1:1000 to 1:2000). Muscarine has received a more detailed study, both on turtles' hearts and on the embryo. Pickering (1893, 1895, 1896) found that the hearts of chicken embryos up to the 140th hour do not react to muscarine, in even stronger concentrations than are effective after the 200th hour. But he found that there was a gradual increase in sensitivity, solutions containing per cubic centimeter 0.5 mgm. being necessary at 185 hours, 0.1 at 200, 0.075 at 250 hours. These concentrations caused complete inhibition, which was only partially removed by atropine in similar concentrations. They were therefore probably physiologically unduly high. It is difficult to compare the concentrations with those affecting adult animals. He found further (1896) that the hearts of rabbit, cat and rat embryos were typically affected by muscarine and atropine, 0.05 mgm. per cc. being effective at the earliest period studied by him, apparently about $9\frac{1}{2}$ days. Kobert (1886) found that the hearts of rabbit and cat embryos just before birth were less sensitive than two weeks afterwards. His methods, however, did not allow of accurate quantitative comparisons. He also found that the hearts of very young polywogs were not affected by muscarine, which as they grew older showed increasing sensitivity up to the adult stage. The period at which Pickering found muscarine sensitivity for chicken embryos, he found also corresponded very well with the period at which cardiac stimulation might produce inhibition. Nerve fibres, however, reach the heart cells at an earlier period. These facts apparently suggest that vagus nerve fibres must at least reach the cell before it becomes sensitive to acetylcholine or muscarine. Vagus fibre nerves never reach the turtle's ventricle and it is not affected by reasonable concentrations. Though the matter perhaps requires further study, the above data also suggest that not only must the nerve fibres reach the cell but they must

become functional before complete sensitivity to muscarine and presumably acetylcholine occurs. In other words, the tonic impulses impinging upon the heart cell change its metabolism and make it normally sensitive. That acetylcholine in concentrations inadequate to affect contractility or rate does change cardiac metabolism, appears to be shown by the work of Gremels (1936). With the removal of these tonic impulses, one might expect the cell to become again non-responsive. Yet this is opposed to the observations of Cattell and Wolff for acetylcholine and of Anderson in regard to pilocarpine. It has already been pointed out that presumably degeneration or even the cessation of tonic impulses produces a change in the cell. However, even if it be accepted that a nerve ending must have reached a cell before it becomes sensitive to the action of acetylcholine and muscarine, it does not necessarily follow that the drug acts on the histological nerve ending. Further, there is the remarkable case (Baur, 1928) of the chicken amnion which has no nerve supply and yet reacts to a concentration of 1 in 7 millions acetylcholine.

Mode of action. Our knowledge of the chemistry of the cell and in many cases of the drugs we use, is so imperfect that even in the best of cases the results obtained by experiment merely justify speculation. This is, however, attempted in this case, though with a recognition of the pitfalls.

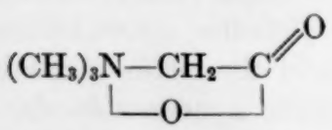
The physiological compound acetylcholine, owing to its relatively simple chemical characteristics and its pharmacological potency, forms the best basis for the study of the mode of attachment and of action of this group. Further, owing to its great physiological importance, it and its chemical relatives have alone been subjected to such searching experimentation as to adequately justify speculation in regard to its mode of action.

Certain results and deductions are fundamental to our inquiry. First, it has been shown by Clark (1926a) that the amount of acetylcholine which unites with the cells of frogs' heart muscle strips to decrease their contractility by one half, is far from being adequate to form a monomolecular layer on the surface of the cells. The physical or chemical affinity of the cell for the drug must be exerted solely by certain elements in the cell or its surface. Clark (1926b) has made a study of the relationship between the acetylcholine concentration and the decrease in contraction of the heart and the rectus abdominis in frogs. He interprets the results as indicating a monomolecular reaction between the drug and some substance on the cell. This decrease

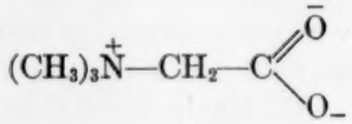
in contraction may not be the sole effect produced by the drug; indeed Gremels' work suggests that it is not, as in his experiments there was a decreased oxygen consumption, even if the work done were the same, and it may well be that these two responses of the cell do not vary with concentration in a parallel fashion. In Gremels' experiments with adrenalin this was not the case, very small concentrations decreasing oxidation, larger increasing, but only still larger affecting cardiac frequency or work done. A study of the maximum decrease in contractility by acetylcholine is complicated, as Mezey and Straub (1936) have shown, by changes in conductivity.

The second fundamental point is made evident from the work of Hunt. Choline and a great many of its derivatives or analogues produce muscarine-like effects. Quantitatively these compounds differ greatly. Betaine, in which the sole change is the transformation of the terminal methanol group into an acid, is without this effect. Yet the esters of this acid group again show the muscarine action (Hunt and Renshaw, 1925–26).

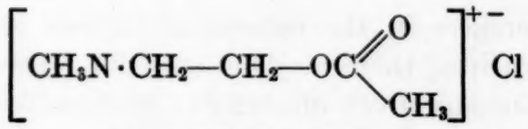
The whole choline group may be looked upon as belonging to the group of quaternary onium compounds. Members of this group, as Ing and Wright (1932–33) have pointed out, are strong electrolytes, their halide salts are very soluble in water but not in lipids and are not appreciably surface active. In accordance with this von Oettingen and Bowman (1933) have shown specifically for choline and certain of its congeners, that they have no influence on surface tension or colloid systems. Nor can they at any pH liberate by dissociation, a lipid-soluble base or ion. Lipoid solubility or the surface tension type of adsorption cannot explain their attachment to the cell. As strong electrolytes they can hardly be adsorbed as possibly weak bases are (Trevan and Boock, 1927; Phelps, 1935), either in the form of the unionized salt or free base. Renshaw (1925, and with Hotchkiss, 1926) has pointed out that all substances of this onium group which produce physiological effects, are ionizable in body pH ranges. At physiological pH values betaine exists largely as the electrically neutral inner salt or zwitter-ion and has no muscarine action, whereas betaine esters under these conditions exist as cations.



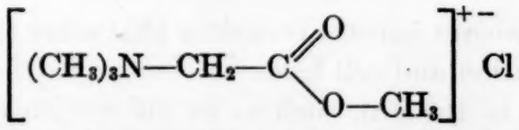
Inner salt of betaine



Zwitter ion of betaine



Acetylcholine chloride



Methyl ester of betaine hydrochloride

These facts, with others, suggest that the reaction of pharmacologically active choline-like compounds with the cell is ionic or salt-like in character, i.e., consist in the cation replacing equivalent amounts of K, Na or Ca ion of the cell constituents. Ing and Wright (1932–33), who studied the curare-like action of onium compounds, also suggest that this type of reaction explains their attachment to the cell. Roepke and Welch (1936), following a suggestion of Ing and Wright, used as a model for the cell various zeolites and showed that choline chloride, acetylcholine chloride and arsenocholine chloride [$(CH_3)_3As \cdot CH_2CHOH$]Cl show this type of cationic exchange and while choline as compared to arsenocholine in equal concentration, showed some differences in ability to displace sodium (85.1 to 81.4) or calcium (48.1 to 32.3) from Na or Ca zeolite, no significant difference was to be found between choline and acetylcholine in this respect. The difference found between choline and arsenocholine hardly seems adequate to explain the differences found by Welch and Roepke (1935) between the pharmacological activity of the compounds, 1000–35, or between the activity of their acetylated forms, 1000–11. Yet it is evident that the physical forces within the molecule which must be considerably different in arsenocholine with the heavy atom As, as compared to the light N of choline, do influence somewhat even this type of reaction. Of course it is evident that the cationic exchange with a cell will not necessarily parallel that of zeolite.

In a study of the affinities of choline acetylcholine and arsenocholine with the specific esterase, Roepke (1936) again showed that the differences in chemical affinity were not great. He had previously shown that they were equally hydrolysed by the enzyme.

The simplest suggestion then appears to be that the primary attachment of the choline group of active substances to the cell is a cationic exchange, so that the cell is deprived of base Na, K or Ca. Owing to

the inherent differences in the cations of various choline analogues (As or P) or derivatives, there may be some differences in the amount of base removed for any given quantity of choline-like cation. These differences appear, however, inadequate to explain their pharmacological potency.

Consequently, we are forced to consider that after their attachment, the complex of cation and cell leads to changes in the whole physicochemical activity of the cell, such as to differ with each complex so formed. We have, indeed, in the studies that have already been made, proofs that such differences occur. Hunt and Renshaw (1934), for instance, report that the action of acetyl-β-methyl choline, which produces a long-continued low blood pressure, is much more resistant to atropine i.e., requires higher doses of atropine to overcome it, than is the case with acetylcholine. The same is true of this drug's effects on the heart. Again, while the attachment to any cell may be a cationic exchange and equally readily produced in cells of different physiological activities, vasodilator or cardiac or ganglionic, the effects produced by the same concentration may be either apparent or not, dependent on the characteristics of the cell. For example, in low concentrations acetylcholine causes vasodilatation and only in higher ones ganglionic cardiac inhibition; and further, the obvious effects of different choline derivatives on different cells will not necessarily appear equal. Chloracetylcholine has 1000 times less muscarine action than acetylcholine, but a similar stimulating effect on ganglia (nicotine effect). According to Simonart (1933), while the muscarine effect on blood pressure ranges from 125 for acetyl, 25 for propionyl and 1 for butyryl cholines, the value of their potency in raising blood pressure after atropine, ganglionic action, was the reverse. While Hunt and Renshaw (1934) do not entirely agree with these values, they also found that their muscarine and nicotine actions were reversed in quantitative values.

Chemically, the most obvious group which might affect pharmacological activity in choline hydrochloride is the terminal hydroxyl in the side chain, but this has undergone modification in many of the compounds studied. Acetylation of the hydroxyl leads to a great increase in muscarine-like effects. As pointed out above in the betaine esters, the hydroxyl does not exist. If an oxyphenyl group be substituted for the hydroxyl, the muscarine activity disappears (Hunt and Renshaw, 1929), but this substance has a marked effect on ganglia. Further, any shortening of the side chain, as in formocholine formula, or length-

ening to a propyl group again increases the toxicity of these compounds for mice and changes their pharmacological muscarine potency, though not in the same proportion.

It is unnecessary to quote the many other examples that could be culled from the literature, but it appears obvious that the side chains are not concerned in the attachment to the cell, but in the changes produced by the complex so formed, on the physiological state of the cell.

This conclusion for the cholines will naturally include muscarine, natural or synthetic, as they are choline derivatives, but cannot be applied to atropine and pilocarpine with entirely different chemical structures and physico-chemical properties. Indeed, the evidence produced by Clark in regard to the antagonism between acetylcholine and atropine, strongly suggests that these drugs reacted with different cell receptors (Clark 1926b). Yet the work of Roepke (1936) on the acetylcholine esterase shows that certain considerations, namely, that the atropine is a competitive inhibitor of the enzyme with an affinity for it 17 times that of acetylcholine, suggests a chemical antagonism instead of one of effect as suggested by Clark. Tropine, on the other hand, with negligible pharmacological effect, has an affinity for the enzyme equal to laevo-hyoscyamine, which would lead to the opposite conclusion, namely, that the cell atropine complex was formed by reaction with different receptors than those involved in the reaction with acetylcholine, and that consequently the effects produced on the cell by union with acetylcholine and atropine were different. But here again cholinesterase cannot be considered as anything but a rough model of the cell itself, though it is probably derived from it and undoubtedly in other cases a part of the cell.

The fact that in the ganglion cell atropine does not affect the action of acetylcholine, may again be cited as evidence that acetylcholine and atropine act on different receptor substances, that for atropine being either absent in this case or unable to interfere with the chain of reactions in spite of its attachment to the cell. The same might explain the effect on the contractile mechanism of the bladder and on the skeletal muscle cell.

The following further speculation may be allowed. Feldberg and Vartiainen (1934) have shown that acetylcholine is produced by the preganglionic nerve ending. If the acetylcholine may be produced by cationic exchange, potassium excess might well set it free, or if the propagation of the impulse is accompanied by a liberation of potassium,

as Cowan (1934) seems to have shown, the same process might well liberate acetylcholine from the endings. Gaddum and Dale (1936) indeed, refer to unpublished evidence that acetylcholine is so liberated from nerves. Some of the acetylcholine so liberated doubtless reacts with the cell and again might displace potassium, as one of its effects. Indeed, Vogt's experiments (1936) go to show that the stimulated ganglion loses potassium. Further, potassium excess might liberate acetylcholine from the nerve ending, as shown by Brown and Feldberg (1936).

REFERENCES

ADLOR. Ztschr. f. klin. Med. **40**: 248, 1900.

AFANASSIEW AND PAWLOW. Pflüger's Arch. **16**: 173, 1878.

ALLES. Physiol. Reviews **14**: 276, 1934.

AMANTEA. Arch. di Fisiol. **22**: 211, 1924.

ANDERSON. J. Physiol. **33**: 414, 1906.

BABKIN. Aussere Sekretion der Verdaungsdrusen. 2nd ed. Springer, Berlin, 1928.

Trans. Roy. Soc. Can. V. **25**: 205, 1931.

BABKIN AND SAWITSCH. Ztschr. f. physiol. Chem. **56**: 321, 1908.

BACQ AND MONIER. Arch. Intern. de Physiol. **40**: 485, 1935.

BARCROFT. J. Physiol. **27**: 31, 1901.

BATTELLI. Arch. Ital. di Biol. **27**: 263, 1897.

BAUER. Deutsch. Arch. f. klin. Med. **107**: 38, 1912.

BAUR. Arch. f. exper. Path. u. Pharmakol. **134**: 49, 1928.

BAYLISS AND STARLING. J. Physiol. **24**: 99, 1899.

BEER. Arch. f. Anat. u. Physiol., suppl. 201, 1892.

V. BERGMANN AND KATSCH. Deutsch. Med. Wchnschr. **39**: 1294, 1913.

BERTEAU. Berl. klin. Wchnschr. 581, 1880.

BEZOLD AND BLOEMBAUM. 1867. Cit. by CUSHNY, 1924, p. 606.

BIJLIMA. Pflüger's Arch. **142**: 531, 1911.

BINZ. Vorlesungen u. Pharmakologie. 2nd ed. Hirschwald, Berlin, 1891.

BLOMBERG AND RONNELL. Arch. Internat. de Pharmacod. **37**: 369, 1930.

BOEHM. Arch. f. exper. Path u. Pharmakol. **19**: 85, 1885.

BRODIE AND DIXON. J. Physiol. **30**: 476, 1904.

BROWN, DALE AND FELDBERG. J. Physiol. **87**: 394, 1936.

BROWN AND FELDBERG. J. Physiol. **86**: 290, 1936.

BROWN AND MCSWINEY. J. Physiol. **61**: 261, 1926.

BULBRING AND BURN. J. Physiol. **83**: 483, 1935.

BUSQUET. Compt. rend. Soc. Biol. **114**: 1083, 1933.

CAMUS AND GLEY. Arch. intern. de Physiol. **13**: 102, 1913.

CATTELL AND WOLFF. Science **82**: 106, 1935.

CHANG. Chinese J. Physiol. **3**: 229, 1929.

CHANG AND GADDUM. J. Physiol. **79**: 255, 1933.

CLARK. J. Physiol. **61**: 530, 1926a; **61**: 547, 1926b.

COWAN. Proc. Roy. Soc. Lond. **B115**: 216, 1934.

CRAWFORD. J. Pharmacol. Exper. Therap. **22:** 1, 1924.
CULLIS AND LUCAS. J. Physiol. **86:** 63. P. 1936.
CURSCHMANN. Berl. Klin. Wchnschr. 353, 1877.
CUSHNY. Arch. f. exper. Path. u. Pharmakol. **31:** 432, 1893.
J. Physiol. **30:** 176, 1903.
J. Physiol. **41:** 233, 1910.
Springer, Berlin, 1924a. Heffter's Handbuch der exp. Pharmakologie, p. 611. II.²
Springer, Berlin, 1924b. Heffter's Handbuch der exp. Pharmakologie, p. 624. II².
Springer, Berlin, 1924c. Heffter's Handbuch der exp. Pharmakologie, p. 632. II².
Springer, Berlin, 1924d. Heffter's Handbuch der exp. Pharmakologie, p. 615. II².
CUSHNY AND PEEBLES. J. Physiol. **32:** 509, 1905.
DALE. J. Pharmacol. Exper. Therap. **6:** 147, 1914.
J. Physiol. **80:** 10. P. 1933.
DALE AND FELDBERG. J. Physiol. **82:** 121, 1934.
J. Physiol. **81:** 320, 1934.
DALE, FELDBERG AND VOGT. J. Physiol. **86:** 353, 1936.
DALE AND LAIDLAW. J. Physiol. **45:** 1, 1912.
DIXON AND BRODIE. J. Physiol. **29:** 97, 1903.
DIXON AND RANSOM. Ergebn. d. Physiol. **12:** 268, 1912.
Heffter's Handbuch. **2:** 792, 1924.
DRESER. Arch. f. exper. Path. u. Pharmakol. **26:** 255, 1889.
ECCLES. J. Physiol. **85:** 179, 207, 1935.
EDMUNDS AND ROTH. Am. J. Physiol. **23:** 28, 1908.
EINTHOVEN. Pflüger's Arch. **51:** 428, 1892.
ENGELHART. Pflüger's Arch. **227:** 220, 1931.
v. ESWALD. Arch. f. exper. Path. u. Pharmakol. **134:** 347, 1928.
EWING. J. Pharmacol. Exper. Therap. **3:** 1, 1911.
FARDON. Biochem. J. **3:** 405, 1908.
FELDBERG AND GADDUM. J. Physiol. **81:** 305, 1934.
FELDBERG AND GUIMARAIS. J. Physiol. **85:** 15, 1935.
FELDBERG AND VARTIAINEN. J. Physiol. **83:** 103, 1934.
FLEMING AND MACINTOSH. Quart. J. Exper. Physiol. **25:** 207, 1935.
FÖHNER. Monatschr. f. prakt. Thierheilk **5:** 336, 1894.
FRANK, NOTHMANN AND HIRSCH-KAUFMANN. Arch. f. Physiol. **197:** 270, 1923.
FÜHNER. Biochem. Ztschr. **76:** 232, 1916.
GADDUM AND DALE. Gefässerweiternde Stoffe der Gewebe. Thieme, Leipzig, 1936.
GAISBOCK. Arch. f. exper. Path. u. Pharmakol. **66:** 398, 1911.
GANTER. Arch. f. exper. Path. u. Pharmakol. **103:** 84, 1924.
GANTER AND STATTMULLER. Ztschr. f. d. g. exper. Med. **42:** 143, 1924.
GASKELL. Phil. Trans. Roy. Soc. 993, 1882.
J. Physiol. **4:** 43, 1883.
GASSER. J. Pharmacol. Exper. Therap. **27:** 395, 1926.
GIBBS AND SZELÖCZEY. Arch. f. exper. Path. u. Pharmakol. **168:** 64, 1932.

GOTTLIEB. Arch. f. exper. Path. u. Pharmakol. **33**: 261, 1894.
GREMELS. Arch. f. exper. Path. u. Pharmakol. **182**: 1, 1936.
HANC. Pflüger's Arch. **73**: 453, 1898.
HANDOWSKY AND PICK. Arch. f. exper. Path. u. Pharmakol. **71**: 89, 1912.
HARNACK. Arch. f. exper. Path. u. Pharmakol. **9**: 152, 1878.
HARNACK AND MEYER. Arch. f. exper. Path. u. Pharmakol. **12**: 366, 1880.
HARNACK AND WITKOWSKI. Arch. f. exper. Path. u. Pharmakol. **5**: 401, 1876.
HEDBOM. Skand. Arch. f. Physiol. **8**: 168, 1898.
HEIDENHAIN. Pflüger's Arch. **5**: 309, 1872.
HEINEKAMP. J. Lab. Clin. Med. **8**: 104, 1922.
HENDERSON. Arch. internat. Pharmacod. **27**: 205, 1922.
J. Pharmacol. Exper. Therap. **21**: 99, 1923.
HENDERSON AND ROEPKE. Canadian Chem. and Metall. **16**: 4, 1932.
Arch. f. exper. Path. u. Pharmakol. **172**: 314, 1933.
J. Pharmacol. Exper. Therap. **51**: 97, 1934; **54**: 408, 1935.
HIRSCHMANN. Arch. f. (Anat. u.) Physiol. 309, 1863.
HUNT AND TAVEAU. Brit. Med. J. **2**: 1788, 1906.
Hyg. Lab. Bull. U. S. A. **73**: 1911.
J. Pharmacol. Exper. Therap. **1**: 303, 1909.
HUNT. J. Pharmacol. Exper. Therap. **6**: 477, 1915.
Am. J. Physiol. **45**: 197, 230, 1918.
HUNT AND RENSHAW. J. Pharmacol. Exper. Therap. **25**: 315, 1925.
J. Pharmacol. Exper. Therap. **29**: 17, 1926.
J. Pharmacol. Exper. Therap. **35**: 99, 1929.
J. Pharmacol. Exper. Therap. **51**: 237, 1934.
ING AND WRIGHT. Proc. Roy. Soc. Lond. **B 109**: 337, 1932; **B 114**: 48, 1933.
IVY. Physiol. Reviews **14**: 1, 1934.
JACOBI. Arch. f. exper. Path. u. Pharmakol. **29**: 204, 1891.
JENDRASSIK AND WILL. Arch. f. exper. Path. u. Pharmakol. **153**: 94, 1930.
JONESCU. Arch. f. exper. Path. u. Pharmakol. **60**: 154, 1909.
JOURDAN AND NOWAK. Arch. internat. Pharmacod. **53**: 121, 1936.
KATSCH. Zentralbl. Bioch. Biophys. **16**: 181, 1914.
KEETON, LUCKHARDT AND KOCH. Am. J. Physiol. **51**: 469, 1920.
KEHRER. Arch. f. Gyneck. **81**: 160, 1907.
KIBJAKOW. Pflüger's Arch. **232**: 432, 1933.
KISCH. Arch. f. exper. Path. u. Pharmakol. **116**: 227, 1926.
KOBERT. Arch. f. exper. Path. u. Pharmakol. **20**: 92, 1886.
KOCHMANN. Arch. internat. Pharmacod. **12**: 99, 1903.
KÖGL, DUISBERG AND ERXLEBEN. Ann. d. Chem. **489**: 156, 1931.
KOROVITSKY. J. Physiol. **57**: 213, 1923.
LAIDLAW. In BARROWCLIFFE AND TUTIN. J. Chem. Soc. **95**: 1966, 1909.
LANGLEY. J. Physiol. **1**: 96, 1878a.
J. Physiol. **1**: 339, 1878b.
J. Physiol. **11**: 623, 1890.
23: 407, 1898.
33: 374, 1906.
LANGLEY AND DICKENSON. J. Physiol. **11**: 123, 1890.
Proc. Roy. Soc. **46**: 423, 1889.

LANGLEY AND ANDERSON. J. Physiol. **12:** 347, 1891.
J. Physiol. **13:** 460, 1892.
J. Physiol. **18:** 67, 1895.
J. Physiol. **19:** 71, 131, 1896.
LANGLEY. J. Physiol. **27:** 224, 1901.
J. Physiol. **43:** 125, 1912.
LANGLEY AND MANGUS. J. Physiol. **33:** 34, 1905.
LAVAGNA. Therap. Monatschr. **9:** 364, 1895.
LEHEUX. Koninklijke Akademie van Wetenschappen Amsterdam, 1919a.
Pflüger's Arch. **173:** 8, 1919b.
Pflüger's Arch. **190:** 270, 1921.
LEYDEN. Berl. klin. Wchnschr. 386, 404, 1877.
LILJESTRAND. Pflüger's Arch. **175:** 111, 1919.
LOEWI. Arch. f. exper. Path. u. Pharmakol. **70:** 351, 1912.
Pflüger's Arch. **193:** 201, 1921.
Pflüger's Arch. **204:** 361, 629, 1924.
LOEWI AND MANSFELD. Arch. f. exper. Path. u. Pharmakol. **62:** 180, 1910.
LOEWI AND NAVRATIL. Pflüger's Arch. **206:** 123, 135, 1924.
LÖWIT. Pflüger's Arch. **28:** 312, 1882.
LUCAS AND HENDERSON. Arch. internat. Pharmacod. **54:** 201, 1936.
LUCHSINGER. Pflüger's Arch. **15:** 482, 1877.
V. LUDANY AND JOURDAN. Arch. internat. Pharmacod. **53:** 281, 1936.
MACINTOSH AND RAWLINSON. Quart. J. Exper. Physiol. **25:** 199, 1935.
MACLEAN. Biochem. J. **3:** 1, 1908.
MAEVSKY. J. Physiol. **57:** 307, 1923.
MAGAARD. Virchow's Arch. **89:** 258, 1882.
MAGNUS. Pflüger's Arch. **102:** 123, 349, 1904.
Pflüger's Arch. **108:** 1, 1905.
Pflüger's Arch. **123:** 95, 1908.
Die Naturwiss. 8, 1920.
MARKOWITZ. Am. J. Physiol. **97:** 271, 1931.
MARMÉ. Therap. Monatschr. **4:** 291, 1890.
MARRIS. Brit. Med. J. **2:** 717, 1916.
Med. Res. Com. Sp. Rept. Series 9, August, 1917.
MARSHALL. J. Physiol. **31:** 120, 1904.
MAY. J. Physiol. **31:** 260, 1904.
MCGUIGAN. J. A. M. A. **176:** 1338, 1921.
MEIER. Biochem. Ztschr. **2:** 415, 1907.
MEZEY AND STRAUB. Arch. f. exper. Path. u. Pharmakol. **182:** 183, 1936.
MODRAKOWSKI. Pflüger's Arch. **114:** 487, 1906.
NIITANI. Arch. f. Ophthal. **121:** 471, 1929.
MÜLLER. Pflüger's Arch. **134:** 289, 1910.
NEUKIRCH. Pflüger's Arch. **147:** 153, 1912.
VON OETTINGEN AND BOWMAN. J. Pharmacol. Exper. Therap. **48:** 333, 1933.
VON OETTINGEN AND MARSHALL. J. Pharmacol. Exper. Therap. **50:** 20, 1934.
OPENCHOWSKI. Arch. f. (Anat. u.) Physiol. 549, 1889.
OTT AND FIELD. J. Physiol. **1:** 193, 1878.

PÄTZ. Ztschr. f. exper. Path. u. Therap. **7**: 577, 1910.
PAWLOW. Pflüger's Arch. **17**: 555, 1878.
PEDDEN, TAINTER AND CAMERON. J. Pharmacol. Exper. Therap. **55**: 242, 1935.
PEWSNER. Biochem. Ztschr. **2**: 339, 1906.
PHELPS. Biochem. J. **29**: 1043, 1935.
PICKERING. J. Physiol. **20**: 165, 1896.
J. Physiol. **14**: 383, 1893.
J. Physiol. **18**: 470, 1895.
PILCHER. J. Pharmacol. Exper. Therap. **52**: 206, 1934.
PLANT AND MILLER. J. Pharmacol. Exper. Therap. **27**: 361, 1926.
PLATZ. Ztschr. f. exper. Med. **28**: 81, 1922a.
Ztschr. f. d. g. exper. Med. **30**: 189, 1922b.
POLLAND. J. Clin. Invest. **9**: 319, 1930.
POOS. Arch. f. exper. Path. u. Pharmakol. **126**: 307, 1927.
PREVOST. Compt. rend. Acad. d. Sci. **79**: 381, 1874.
RENSHAW. Science **62**: 385, 1925.
RENSHAW AND HOTCHKISS. J. Am. Chem. Soc. **48**: 2698, 1926.
RHODE AND OGAWA. Arch. f. exper. Path. u. Pharmakol. **69**: 201, 1912.
RHODIUS AND STRAUB. Pflüger's Arch. **110**: 492, 1905.
RIEGEL. Ztschr. f. klin. Med. **37**: 381, 1899.
ROEPKE. In press, 1936.
ROEPKE AND WELCH. J. Pharmacol. Exper. Therap. **56**: 319, 1936.
ROSENBLUETH AND CANNON. Am. J. Physiol. **108**: 384, 1934.
ROTHBERGER AND WINTERBERG. Pflüger's Arch. **132**: 233, 1910.
RUDOLF AND BULMER. Am. J. Med. Sci. **168**: 641, 1924.
SAWITSCH. 1899. Cit. by BABKIN, p. 779, 1928.
SAWITSCH AND SSOSHENSTWENSKIJ. Ber. d. g. Physiol. **15**: 78, 1922.
SCHEPOWALNIKOW. 1899. Cit. by BABKIN, p. 779, 1928.
SCHMIEDEBERG. 1870. Cit. after CUSHNY, p. 608, 1924.
SCHMIEDEBERG AND HARNACK. Arch. f. exper. Path. u. Pharmakol. **6**: 101, 1877.
SCHMIEDEBERG AND KOPPE. 1869. Cit. KOBERT, 1886.
VON SCHROFF, FRÖHLICH AND LICHTERFELS. 1852. Cit. by CUSHNY, p. 606, 1924.
SCHÜTZ. Arch. f. exper. Path. u. Pharmakol. **21**: 341, 1886.
SIMONART. J. Pharmacol. Exper. Therap. **46**: 157, 1933.
SUBBOTIN. Arch. f. klin. Med. **6**: 285, 1869.
STEWART AND ROGOFF. J. Pharmacol. Exper. Therap. **17**: 227, 1921.
SZPILMANN AND LUCHSINGER. Pflüger's Arch. **26**: 459, 1881.
TITELBAUM. Ztschr. f. d. g. exper. Med. **52**: 408, 1926.
TOBY. Quart. J. Exper. Physiol. **26**: 45, 1936.
TRENDELENBURG. Arch. f. exper. Path. u. Pharmakol. **69**: 79, 1912; **81**: 55, 1917.
TREVAN AND BOOCK. Brit. J. Exper. Path. **8**: 307, 1927.
TSEHEBOKSAROFF. Pflüger's Arch. **137**: 59, 1911.
TWEEDY AND RINGER. Lancet **1**: 795, 1880.
UNGER. Pflüger's Arch. **119**: 373, 1907.
USCHAKOW. 1896. Cit. by BABKIN, p. 321, 1928.
VELLA. Maly's Jahresber. **11**: 300, 1881.

VOGT. J. Physiol. **86:** 258, 1936.
WEBER. Centralbl. Med. Wissensch. **14:** 769, 1876.
WEBER. Arch. f. (Anat. u.) Physiol. 63, 1914.
WELCH AND ROEPKE. J. Pharmacol. Exper. Therap. **55:** 118, 1935.
WINKELSTEIN AND ASCHNER. Am. J. Med. Sci. **168:** 812, 1924.
WINTERBERG. Ztschr. f. exper. Path. u. Therap. **4:** 636, 1907.
ZITOWITSCH. 1902. Cit. by BABKIN, p. 400, 1928.
ZUNZ AND TYSEBAERT. J. Pharmacol. Exper. Therap. **8:** 325, 1916.

PHYSICAL FACTORS INVOLVED IN THE ACTIVITIES OF THE MAMMALIAN KIDNEY

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The analysis of the physical factors affecting urine formation has received an immense impetus from observations on the isolated kidney, perfused at constant blood flow (Richards and Plant, 1922a) or at constant arterial pressure as in the heart-lung-kidney preparation (Verney and Starling, 1922). It is the purpose of the present review to give an account of those physical properties of the kidney which appear to be important in determining urine formation in the isolated kidney, and to attempt to assess the evidence available to indicate their probable significance in the intact animal in health and disease. It is a further purpose to outline the general theory relating the chief physical properties of the kidney, to point out certain major gaps in our knowledge, and to indicate the points in the evidence which have necessitated recent changes in the theory. No comprehensive account of all physical properties of the kidney has been contemplated; for example, no systematic account will be given of the effects of temperature changes (Conway, 1925; Bickford and Winton, 1933, 1937), of heat production (Jannsen and Rein, 1928), or of action potentials (Dungern, 1935). The properties which will chiefly be discussed may be summarized by the headings glomerular pressure, intrarenal pressure, leakage from the tubules, and the factors determining the blood flow. Since the mammalian kidney has defied attempts to apply the methods of direct microscopic observation (Ebbecke and Leach, 1933) which Richards and his school have developed with such fruitful consequences in the frog (Richards, 1929, 1935; Ekehorn, 1931), the physical analysis of the mammalian kidney depends on the relatively indirect and complicated business of discovering the relations between those flows—urine and blood—, and those pressures—arterial, venous, ureter, and extrarenal—, which are accessible to accurate measurement. An additional flow which must be determined if anything like a comprehensive analysis is to be possible is the rate of glomerular filtration. Successive approximations to a method for measuring it have appeared, for example, sulphate

clearance (Mayrs and Watt, 1922), creatinine clearance (Rehberg, 1926), xylose clearance (Jolliffe, Shannon and Smith, 1932) and inulin clearance (Shannon, 1935a, b). Though the last method appears well authenticated in the conscious animal, it is probably inapplicable to the isolated kidney of the dog (Shannon and Winton, 1937) because at low urine flows the inulin clearance is substantially greater than the creatinine clearance. The volume of the kidney is a variable which one would like to be able to include in the analysis; it enjoyed great popularity among early renologists who were concerned to record qualitative changes; the volume of the kidney increases independently with both diuresis and increase in blood flow, the former being probably the more important quantitatively; it also increases with increase in ureter pressure or venous pressure and with decrease in extrarenal pressure. These relations have not yet been worked out quantitatively, and the discussion of renal volume and its changes is, therefore, outside our present purpose. A perusal of general accounts of renal physiology, such as Ellinger (1929), Berglund and Medes (1935), and Newton (1936), will make it clear that the present survey is concerned only with one aspect of the subject. The enquiry into this aspect, that of the physical properties, is only just beginning, and has consequently been mainly concerned with the simplest preparation of the kidney, the heart-lung-kidney introduced by Bainbridge and Evans (1914), and its even simpler modification, the pump-lung-kidney first used by Jacobj (1928), but first used successfully and in its modern and less elaborate form by Hemingway (1931). The description "isolated kidney" in what follows refers to one of these preparations, in both of which the arterial pressure can be kept accurately constant. Observations at constant blood flow are more difficult to interpret than those at constant arterial pressure and are much less numerous; when an experiment conducted at constant blood flow is under discussion, e.g., Richards and Plant (1922a, b), the fact will be specified.

Ureter pressure. Ludwig (1861) described the reduction of urine flow produced by an increase in ureter pressure, and attributed it to a reduction of the filtration pressure across the glomerular membrane produced by a damming back of the fluid in the tubules. Heidenhain (1883) agreed with his description, but questioned his interpretation on the grounds that the hypothesis of reabsorption in the tubules implied that only a small fraction of the glomerular filtrate reached the ureter, the pressure in which might be independent of that in the glomerulus. Cushny (1902) similarly rejected Ludwig's hypothesis as incredible,

arguing that "if the resistance to the escape into the ureter be increased, the natural result is that more of the fluid is deflected from this route." Henderson (1905) working in Starling's laboratory supported Ludwig's view, and showed that under the conditions in which the minimum difference between arterial and ureter pressures were obtained, this difference amounted to little more than the value of the colloid osmotic pressure shown by Starling (1899) to result from the presence of the plasma proteins. Brodie and Cullis (1906) argued that the objections to Ludwig's hypothesis were unsound in principle, because whereas glomerular filtration was controlled by small hydrostatic pressures, reabsorption in the tubules was in any case conducted against large osmotic gradients. The disproportion between the small ureter pressures concerned and these osmotic gradients was illustrated by an experiment in which a rise in ureter pressure of only 15 mm. Hg reduced the urine flow, and increased its total osmotic pressure from 4710 to 7430 mm., an increase of 2720 mm. Hg. They also showed, however, that in decerebrate dogs, a small rise in ureter pressure may increase urine flow, though a large rise always reduces it, and inferred an hypothesis of glomerular and tubular secretion; but Cushny and others showed later that this increase in urine flow with a small rise in ureter pressure does not occur in anesthetized animals, and can be explained by some reflex response to the pressure stimulus in the ureter. Later Cushny (1917) appears to have come round to Ludwig's hypothesis concerning the action of ureter pressure.

The simplest way of discovering whether the reduction in urine flow due to a rise in ureter pressure is due entirely to a change in glomerular activity, or in part also to a change in the reabsorptive activities in the tubules, is to compare the composition of the urine so formed with that of urine from an exactly similar kidney with the urine flow decreased by exactly the same amount but in this case by lowering the arterial pressure. In principle, the same method and argument was used by Marshall and Kolls (1919) who neutralized the diuresis in an anesthetized dog, due to section of the splanchnic nerve, by partial obstruction to flow in the renal artery of the same kidney; the composition of the urine was now the same as that from the control kidney unaffected by either of the two procedures. Verney and Winton (1930) applied a similar method to the heart-lung-kidney, and extended its principle in the double-heart-lung double-kidney preparation, where the diuresis in one kidney due to caffeine was matched with a diuresis in the other kidney induced by increase in arterial pressure; it was

found that at equal urine flows, the composition of the urines was the same, the degree of parallelism of two such kidneys, not treated differently, having been shown to be adequate for the purpose (Canny Verney and Winton, 1930). The latter principle was applied to the heart-lung-double-kidney preparation to see whether the rise in ureter pressure acted on the composition of the urine exactly like a fall in arterial pressure or not (Winton, 1931a). The concentrations of chloride and urea were found to be indistinguishable when the rates of urine flow were matched, and consequently Ludwig's hypothesis that a rise in ureter pressure affects the glomerular filtration rate but not the rate of tubular reabsorption was proved. In view, however, of the important theoretical developments which have since come to rest, in part, on this conclusion, the experiments should be confirmed and extended to other constituents of the urine; for it is possible that the permeability of some part of the tubule may change when the wall of the tubule is stretched by ureteral obstruction (Brodie and Mackenzie 1914); but the evidence so far available is against it.

Venous pressure. Ludwig's (1861) explanation of how an increase in venous pressure reduces the urine flow, is best described perhaps in Heidenhain's (1883) excellent exposition of his work; Heidenhain confirms Ludwig's view that an increase of pressure in the veins so distends the venules that the tubules lying among them are narrowed or obliterated, a view based on histological observation after ligature of the vein, and on "hydraulic" experiments with dog's kidneys. The main effect of a rise of venous pressure, then, is to transmit the pressure to the tubules, and so to act as a back-pressure reducing the rate of glomerular filtration, just as does a rise in ureter pressure. A second effect of obstruction to the outflow of blood, recognized by Ludwig, is to raise the blood pressure throughout the vascular system of the kidney, and hence in particular in the glomerular capillaries. This associated rise in glomerular pressure is, however, only a fraction of the applied venous pressure, the whole of which may be transmitted to the liquid in the tubules; it, therefore, only in part neutralizes the primary obstructive effect on the urine flow of the rise of pressure in the veins.

It is well known that Heidenhain (1883) chose the anuric effect of venous obstruction as crucial evidence dissociating the effects of glomerular capillary pressure and blood flow, showing that the latter and not the former was the effective determinant of the urine flow. He argued that this indicated a secretory activity of the glomerular membrane, dependent upon adequate nutrition and hence upon the blood

flow rather than the blood pressure. Richards and Plant (1922a), Dreyer and Verney (1923), and others, have now shown beyond doubt that Heidenhain was wrong in this interpretation of the facts, and that it is really the glomerular pressure and not the blood flow which controls the urine flow. Perhaps the most clear-cut decision between these views of Ludwig and Heidenhain about the effects of venous pressure is given by the consequences of raising the venous pressure to a given value in a kidney already secreting against a ureter pressure of the same value. The reduction in blood-flow due to the venous obstruction is about the same in such a kidney and in a kidney with unobstructed ureter; hence, according to Heidenhain's view, the urine flow should fall when the venous pressure is raised; but according to Ludwig's view the urine flow should rise, for the obstructive effect of venous pressure on the urine flow should vanish in a kidney with preexisting obstruction applied through the ureter; only the secondary action, that of raising the glomerular pressure, should have manifest consequences. Experiment unequivocally favours Ludwig's view (Winton, 1931b). The complementary experiment on the influence of ureter pressure on a kidney with the venous pressure already raised, shows that the urine flow is quite unaffected by changes of ureter pressure unless this pressure exceeds the venous pressure. This also accords with Ludwig's view.

Heidenhain's view that the reduction of urine flow with rise of venous pressure is primarily due to a reduction in blood flow rather than to pressure changes is, however, still held, for example, by Richards and Plant (1922a), Cushny (1926), and Richards (1935) who emphasize the "stagnation" of blood produced by a rise of venous pressure; the emphasis is now laid not so much on the nutritional aspects of an adequate blood flow, though this aspect of the maintenance of the normal permeability of the glomerular membrane is not ignored, but on the rise of colloid osmotic pressure and of the viscosity of the blood leaving the glomeruli. It is clear that these factors set a limit to the rate of glomerular filtration which could occur in a kidney, as discussed in the section on blood-flow below; it is not clear, however, that the secreting kidney is operating so close to the limits set by these factors that the relatively small reduction in blood flow associated with an effective rise in venous pressure is likely to push the conditions beyond this limit. Quantitative observations are available on the isolated kidney of the dog showing that these factors are not relevant to the anuric action of venous pressure. The experiment quoted above is one; it shows that a

rise in venous pressure together with the associated "stagnation" of blood (about 20 per cent reduction in blood flow) can fail to reduce the urine flow in the presence of a preëxisting ureter pressure. Another observation with the same implication is that a suitable concentration of adrenaline may produce up to 20 per cent reduction in blood flow with increase in urine flow (Winton, 1931d), whereas venous pressures producing reductions in blood flow even considerably less than this will reduce the urine flow. Moreover, a five-fold diuresis due to cooling is accompanied by a halving of the blood flow. It is possible that a comparable analysis of the action of venous pressure in the intact animal, in which creatinine and inulin clearances are higher than in the isolated kidney, will give different results, but the evidence now available does not justify the view that the change of blood flow is the primary factor reducing the urine flow when the venous pressure is raised.

Another possibility that arises, however, is that an increase in venous pressure affects the activity or permeability of the tubules. This can be tested by the principle detailed in connection with the action of ureter pressure, namely, by matching the actions of venous pressure and of a fall in arterial pressure on the urine flow, and comparing the compositions of the urines so formed. In the heart-lung-double-kidney preparation both the chloride and urea concentrations in such urines were found to be indistinguishable (Winton, 1931b), and until further evidence is available it may be taken that venous pressure produces no detectable change in the action of the tubules. Venous pressure differs from ureter pressure in that it produces a fall in blood-flow about proportional to the change in pressure-head. Ureter pressures up to the 20 or 30 mm. needed to stop urine flow in the unstimulated kidney, produce no detectable change in blood-flow; the high ureter pressures observable during diuresis may, however, produce a small fall in blood-flow.

Glomerular pressure. The relations between ureter pressure, venous pressure and urine flow may thus be summarized by saying that the rise in venous pressure which induces a given fall in urine flow always exceeds the rise in ureter pressure which would produce the same fall; this difference is due to the partial obstruction to the outflow of blood which is produced by the venous pressure but not by the ureter pressure; the obstruction and the resulting damming back of blood produces a rise in glomerular capillary pressure. If the venous pressure (V) and the ureter pressure (U) are so chosen that, taken one at a time, they produce equal reductions in the urine flow, we may, therefore, approxi-

mately equate the increment in the glomerular pressure (ΔG) associated with the rise in venous pressure to the difference ($V - U$). If it be, for the moment, supposed that a rise in venous pressure results in an increment in pressure in each part of the vascular system of the kidney, diminishing proportionately from vein to artery, where the arterial pressure (A) is kept unchanged, as it might in a network of rigid tubes traversed by a homogeneous liquid, then the increment in glomerular pressure (ΔG), due to the rise in venous pressure (V) must be $\frac{A - G}{A} \cdot V$. Hence equating the two expressions for ΔG we have:

$$\frac{A - G}{A}, V = V - U$$

or

$$\frac{\text{glomerular pressure}}{\text{arterial pressure}} = \frac{\text{ureter pressure}}{\text{venous pressure}}$$

when the ureter and venous pressures are so chosen that, taken one at a time, they will produce the same reduction in the urine flow (Winton, 1931c).

There are at least two explicit assumptions in the derivation of this equation: 1, that the whole venous pressure is transmitted as an effective back-pressure to the liquid in the tubules; this is rendered likely by the observation that the ratio of ureter to its equivalent venous pressure is independent of the degree of reduction of urine flow produced by them; 2, that blood in the vessels of the kidney behaves like a homogeneous liquid in rigid tubes; this cannot be entirely true, and the question arises whether the deviation from the truth is quantitatively large enough to invalidate the assumption. The evidence bearing on this is as follows: *a*, large blood vessels are undoubtedly distensible, but as the fraction of the whole arterio-venous pressure fall which takes place in the large vessels is so small, a change in this fraction has negligible effects on the pressure distribution compared with changes in smaller vessels; *b*, distensibility of the small vessels which are mainly responsible for the distribution of pressure in the vascular tree has been inferred from pressure-flow curves in perfused organs, in which resistance to flow decreasing with rise of pressure is found. Experiments of this kind, e.g., Fleisch (1919, 1920) are usually performed with Ringer perfusion resulting in edema of the organ, and consequent pressure on the venous outflow; in an abnormal system of this kind the

resistance to flow would decrease with rise of pressure even if the vessels were not distensible. Whittaker and Winton (1933), adapting Starling's (1896) suggestion for avoiding edema of the hind-limb of the dog by allowing Ringer perfusion to interrupt blood perfusion for only a few minutes at a time, found the pressure-flow curve of Ringer's solution linear, and passing through or near the origin. The vessels that are significant in determining the major resistance to flow are, therefore, not detectably distensible within physiological ranges of arterial pressure. Blood-pressure-flow curves, on the contrary, show the well-known reduced resistance at increased pressure, but this as Hess (1907, 1912) and Bayliss (1933) have shown, is also true of blood flowing in rigid glass tubes, and is concerned with the anomalous viscosity of blood, and the effects of velocity of flow on it. Whittaker and Winton (1933) measured the magnitude of this effect on the hind-limb, and found it only became of quantitative significance at very low arterial pressures or abnormally high corpuscular concentrations. It is to be regretted that observations, comparable to those on the hind-limb, have not been made on the kidney; it appears, however, that the technical difficulties in inducing a kidney to undergo accurately reversible changes when its perfusion medium undergoes such drastic changes as from defibrinated blood to serum, are very much more formidable than with the hind-limb. Innumerable papers have been published on observations on the Ringer-perfused mammalian kidney, e.g., Sollmann (1903, 1905), Hühne (1916), Fleisch (1919), and v. Uexküll (1935), many of these claim to have demonstrated some property of the kidney which throws light on the resistance to blood flow in the vascular system of the kidney; scrutiny of the figures shows that the Ringer flows vary from a few per cent up to at most 50 per cent of the blood flow that would have been expected in similar kidneys; this is to be contrasted with the flow, larger than that of blood, which the lower viscosity of Ringer's solution would have led one to expect. The low value of the Ringer flow is apparently not due to vaso-constriction but to the phenomenal value to which the intrarenal pressure rises directly Ringer's solution is substituted for blood, the kidney immediately swelling up and becoming extremely tense (Whittaker and Winton, 1933). The pressure-flow relations in the Ringer-perfused kidney are, therefore, concerned mainly with the factors determining the intrarenal pressure when this is well outside its physiological range, and have no relevance to those changes in size of the vessels which Fleisch (1919) has inferred.

In the absence of direct evidence on the kidney, it is perhaps permis-

sible to assume as an approximation that those vascular elements which are chiefly responsible for the pressure distribution in the kidney behave substantially like rigid tubes as in the hind limb, when they are submitted to small changes of pressure within them, and that the anomalous viscosity of blood introduces no significant error. The observation that the ratio of the ureter pressure to the venous pressure, which reduces the urine flow equally, does not increase with the degree to which the urine flow is reduced is further evidence in favour of the assumption that the increment in the glomerular pressure is proportional to the venous pressure which produces it. The law relating glomerular pressure and the three measurable pressures named above is, therefore, based on assumptions which are reasonably well founded, though far from being proved. Its application to the measurement of glomerular pressure can yield only approximate values, and even these values may be wrong if some hitherto unsuspected property of the kidney should be intervening to make them so. For example, intrarenal pressure was at first supposed not to obstruct the outflow of blood (Winton, 1933), but has now been shown, when high enough, to produce some such obstruction (Herman and Winton, 1936). In kidneys with an intrarenal pressure which obstructs the outflow of blood an error would be introduced into the measurement of glomerular pressure, and this, unless allowed for, would invalidate inferences about the effects of diuretics like urea and sulphate on the glomerular pressure, because they produce increases in the intrarenal pressure. Nevertheless, until an alternative method of estimating the glomerular capillary pressure in the mammalian kidney becomes available, the method of estimation based on the ureter, venous pressure ratio mentioned above may perhaps be adopted provisionally, with a view to discovering whether its consequences are compatible or incompatible with other known properties of the organ. In the heart-lung-kidney, the glomerular pressure so calculated is usually about two-thirds of the pressure in the renal artery, and this value accords well with those guesses (e.g., Brodie, 1914) which have been made on the basis of histological observation. Hayman (1927) found the glomerular pressure in the frog to average about 54 per cent that in the aorta.

The great theoretical importance of a knowledge of the glomerular capillary pressure has induced attempts to evaluate it by measuring the minimum arterial pressure consistent with urine flow. The minimum arterial pressure was found to be 40 to 50 mm. Hg by Ustimowitsch (1870), 30 mm. by Grutzner (1875), and 40 mm. by Starling and Verney

(1925); Tammann (1896) allowed about 20 per cent for the fall of pressure between the renal artery and the glomerular tuft. In the absence of diuretics, the minimum arterial pressure is, however, commonly about 75 mm. Hg (Jannsen and Rein, 1928; Winton, 1934 b), and unless all substances increasing urine flow, including urea, sulphate, and cyanide, are to be supposed to produce very large increases in glomerular pressure, it is clear that the minimum arterial pressure bears no simple relation to the glomerular capillary pressure. The maximum ureter pressure has been regarded as an indication of the pressure in the intra-capsular space since the time of Ludwig (1861); later, when the pressure-fall across the glomerular membrane had been recognized as equal to the colloid osmotic pressure of the plasma proteins, there being no transference of liquid across the membrane, the maximum ureter pressure appeared to provide another method for evaluating the glomerular capillary pressure. Maximum ureter pressures of many values are on record, for example Loebell (1849) 7 to 10 mm. Hg; Hermann (1859) 40 to 60 mm; Heidenhain (1883) 64 mm.; Gottlieb and Magnus (1901) and Henderson (1905) 66 mm.; Ozorio de Almeida (1927) 120 mm. Hg. In the heart-lung-kidney with no diuretic agent in the blood the maximum ureter pressure is commonly about 30 mm. Hg at an arterial pressure of 120 mm.; this maximum ureter pressure can be raised to about 70 mm., without changing the arterial pressure, by cyanide (Starling and Verney, 1925), urea (Winton, 1934 b), cold, or almost any other agent which is capable of producing a large increase in urine flow. If we are to suppose that the maximum ureter pressure is a measure of the intra-capsular pressure, it is evident that the glomerular pressure is exceedingly variable and greatly increased by all the diuretic agents named; but this is contrary to other evidence that these agents act mainly on the tubules, and no confidence can, therefore, be placed on such a method of evaluating the glomerular pressure.

Variations in glomerular pressure independent of that in the renal artery. That differential constriction of the vasa afferentia and vasa efferentia might produce changes in glomerular pressure even if the arterial pressure remained unchanged was foreseen by many of the early workers on the kidney from the time of Ludwig (1861) onwards. It was clearly enunciated by Starling (1912) in the following terms: "A dilatation of the afferent vessels and a slight constriction of the efferent vessels would cause a considerable rise in pressure in the glomerular capillaries, and a consequent increased transudation, without neces-

sarily altering to any marked extent the total circulation of blood through the whole organ. The changes in the afferent and efferent vessels are, however, beyond our control or powers of observation, so that it is impossible to devise at the present time any crucial experiment which might decide the nature of the process occurring in the glomeruli." Richards (1935) claims that "the first experimental evidence . . . in support of the thought . . . . that intra-glomerular pressure is susceptible of regulation by changes in the relationship of the calibre of the afferent and efferent vessels" is provided by the observations of Richards and Plant (1922 a) on the perfused rabbit's kidney. Perfused with hirudinized blood by a pump arranged to deliver a constant output against varying resistances in the kidney, vaso-constriction induced by adrenaline and in other ways produced a large (often 3- to 4-fold) increase of pressure in the renal artery, and some increase in urine flow. This showed that either the vaso-constrictor agents were inhibiting reabsorption of water in the tubules, or more probably that the vasa efferentia were taking part in the general vaso-constriction and consequently that the glomerular capillary pressure was rising, if not by as much as the rise in pressure in the artery, at least by some fraction of it. The experiment repeated (Richards and Plant, 1922 b) with the kidney in an oncometer showed that adrenaline in suitable concentration might produce an increase in volume in the kidney associated with the same order of rise in arterial pressure. These kidneys were secreting no urine, and Richards holds that the increase in volume cannot, therefore, have been due to the secretion of urine and that it must, therefore, have been due to vaso-dilatation. Since adrenaline produced a reduction in volume of similarly perfused forelimbs, the increase in volume in the kidney is, it is suggested, due to the peculiar arrangement of the blood vessels associated with the glomeruli, and hence presumably to dilatation of the vasa afferentia. Two alternative interpretations of these experiments, however, suggest themselves. First, the threefold increase in arterial pressure may have distended some of the larger arteries with relatively large capacity but little influence on pressure distribution, so producing the apparently quite small increase in volume of the kidney (the volume records are not calibrated); if so, the fact that this effect did not appear in the limb experiment was due to the much smaller rise in pressure (50 per cent) or to different compliances of the arteries of the kidney and limb. The second and perhaps more likely explanation would be based on the impression that the changes in volume of the kidney are more often concerned with diuresis than with vascular changes; Richards and

Plant (1922 b) say that their kidneys failed to form urine because the circulation through them had been interrupted during transference of the organ from the animal to the perfusion circuit; but it is well known (Marshall and Crane, 1923, et al.) that recovery of the urine flow follows interruption of the circulation for periods at least up to 15 minutes, and it seems incredible that the transference should have taken longer than this. The records suggest the possibility that urine flow was absent on account of the low perfusion pressure (about 40 mm.), that glomerular filtration began during the short-lived rise in pressure due to vaso-constriction, producing increase in volume in the kidney, and that reabsorption of most of the filtrate in the tubules when the pressure had fallen resulted in the subsequent decrease in volume. Even if the kidneys were in such a condition that they were unable to secrete urine at normal arterial pressures, as the authors imply, changes in the rate of glomerular filtration may have played a part, for reasons given in the section below on "Leakage from the tubules."

These experiments on kidneys perfused at constant blood flow would seem, therefore, to show that the vasa efferentia are capable of constriction and dilatation; it is not so clear that they show that there is a differential action of the agents used on the vasa afferentia and the vasa efferentia. Perfusion of dog's kidneys at constant arterial pressure provides, however, an exceptionally convenient preparation for studying vaso-constrictors such as adrenaline, for in the heart-lung-kidney low concentrations of adrenaline induce substantial diuresis together with a small reduction in blood flow without the complication of a rise in arterial pressure. The increase in urine flow, often between two and tenfold, lasts apparently as long as the adrenaline concentration in the blood is maintained. Higher concentrations of adrenaline produce a reduction in urine flow together with a more profound reduction in blood flow. Applying the ureter pressure, venous pressure ratio discussed above, the changes in glomerular pressure accompanying adrenaline diuresis were found to involve rises, from the normal of 60 per cent of the arterial pressure, to values ranging up to a maximum of 90 per cent; the reductions in urine flow due to higher concentrations of adrenaline were accompanied by a glomerular pressure ranging down to 30 per cent of the arterial pressure (Winton, 1931d).

If it be supposed that the action of adrenaline is entirely confined to the blood vessels and not concerned with tubular activity, it should be possible to follow the changes in glomerular pressure it induces in a simpler way, namely, by following the changes in ureter pressure needed

to keep the urine flow constant. This method, and the more elaborate procedure involving ureter and venous pressure ratios, when simultaneously applied, yield results which are in very fair agreement as to the magnitude of the rise and fall of glomerular pressure at constant arterial pressure, which accompanies the diuretic or anti-diuretic effects of suitable doses of adrenaline. Comparable experiments with diuretics acting mainly on the tubules, such as urea, show a wide divergence between the glomerular pressure estimated from the ureter, venous pressure ratio which remains practically unchanged, and the ureter pressure needed to keep the urine flow constant, which undergoes a large increase.

The simultaneous dilatation of the vasa afferentia and constriction of the vasa efferentia, predicted by Starling, can be shown to accompany the rise in glomerular pressure due to a diuretic dose of adrenaline. This rise implies a diminution of the pressure-fall from the renal artery (where the pressure is constant) to the glomerulus; the diminution amounted, in a typical experiment, to 60 per cent. At the same time the measured reduction in blood flow was only 18 per cent. Hence there was dilatation in the vessels preceding the glomeruli, presumably mainly in the vasa afferentia. The reduction in the blood flow accompanying a rise in glomerular pressure, however, indicates simultaneous constriction of the vasa efferentia (Winton, 1931 d).

The attempt to show that this variation in glomerular pressure independently of the arterial pressure is of real physiological significance and not peculiar to perfused kidneys, has inspired a number of attempts to demonstrate diuretic effects of small doses of adrenaline and other vaso-constrictors in anesthetized animals (Richards and Plant, 1922 c; Mendenhall, Taylor and Richards, 1924; Livingstone, 1928). From these papers it appears that in a few experiments the effect is obtained but in most it is not. I was able to confirm this rather unsatisfactory conclusion in a series of unpublished experiments on the action of adrenaline on anesthetized dogs.

Extrarenal pressure. A number of observers have investigated the effect of raising intra-abdominal pressure on the urine flow. Thorington and Schmidt (1923) found that 15 to 30 mm. Hg produced a progressive reduction in flow under luminal anesthesia. Griffith and Hansel (1925) claim that a pressure of 10 to 20 cm. of water round the human abdomen may induce up to 150 per cent rise in urine flow. Theobald and Verney (1935 b) by intraperitoneal injection of liquid paraffin in conscious dogs showed that 10 to 18 mm. Hg intra-abdominal pressure

had no effect on the course of water diuresis; rather greater values of the pressure inhibited water diuresis, but it was suggested that this might be due to psychological or afferent nerve stimuli rather than to mechanical factors (Theobald and Verney, 1935 a). These authors also showed that a small increase in venous pressure (less than 6 mm. Hg) produced by partial obstruction of the inferior vena cava had no influence on the course of water diuresis, whereas an increase in ureter pressure of only 5 mm. diminished the urine flow if this was small, though even 60 mm. Hg ureter pressure was not enough to abolish urine flow at the height of water diuresis. It is not easy at the moment to bring these observations into direct relation with those on the isolated kidney, in order to assess what part of the effects of intra-abdominal pressure may be attributed to mechanical action, and what part to compensatory responses. Water diuresis cannot be produced in the isolated kidney or even in the deeply anesthetized animal. In the absence of diuresis, and during urea diuresis, the isolated kidney submitted to increased extrarenal pressure exhibits always a reduction in urine flow and in blood flow, comparable with that produced by increase in venous pressure—the greater the applied pressure, the greater the reduction in flow. Extrarenal pressure differs, however, from venous pressure, in that even a small rise in the former produces a definite reduction in the urine flow (Herman and Winton, unpublished). One might have expected that this response would occur in the kidney *in situ* if similar stimuli—venous pressure or intra-abdominal pressure—were applied. Theobald and Verney's demonstration that this is not so, indicates, therefore, the existence of a mechanism which can compensate for the obstruction, and since this comes into action immediately, one would suspect a reflex rise in glomerular pressure. Such a speculation is, however, not supported by the finding of Klisiecki, Pickford, Rothschild and Verney (1931), Rhoads, van Slyke, Hiller, and Alving (1934), or Theobald and Verney (1935 a) that denervation of a kidney in a conscious dog has no detectable influence on the urine flow during water diuresis, or on its inhibition by muscular exercise, emotion or painful stimulation. The effects of intra-abdominal pressure in conscious animals whose kidneys have been denervated have not yet been studied.

Intrarenal pressure. The idea of intrarenal pressure as an important property of a kidney appears to have developed more or less independently in the three sets of circumstances in which its manifestations are at their most striking. First, surgeons noticed the tenseness of kidneys swollen with disease, and inferring that this pressure obstructed

the outflow of urine attempted to relieve the condition by decapsulation. In many cases the attempt appears to have succeeded in establishing an increased urine flow; for example, Harrison (1896) decapsulated cases of acute glomerular nephritis, and many others, such as Eppinger (1921), have studied intrarenal pressure under similar conditions. Fuchs (1931) describes the increased tension in the capsule in acute nephritis, and a bulging of the parenchyma which occurs if a hole is made in the capsule; the cortex is in these circumstances cyanotic before decapsulation and red after it. Fuchs infers that intrarenal pressure due to disease primarily compresses the veins and obstructs the venous outflow. This is perhaps supported by the observations of Hayman (1929) that the resistance to perfusion of a kidney is increased in acute glomerular nephritis. It is claimed, moreover, that decapsulation increases the circulation through the kidney of the dog (Hülse and Litzner, 1926; Stropeni, 1925).

The second set of circumstances in which intrarenal pressure has impressed its importance upon observers occurs during the action of diuretics on anesthetized animals. Such a recognition of tenseness was, of course, implicit in the numerous oncometric researches showing the increase in volume due to diuretics. Brodie (1914) formulated the matter explicitly "If we examine a kidney at the height of diuresis we always find it very hard and tense. The capsule is distended to its fullest degree. If we attempt to make such a kidney expand still further by temporarily clamping the vein we fail completely. We see then that some of the tension set up by the blood-pressure in the glomeruli is transmitted through the capsule wall and the walls of the tubules to the general renal tissues. I made some attempts to measure (the general tension of the kidney substance within its capsule) during active diuresis, but at present have not obtained any very accurate results. As far as they go they indicate a tension of about 40 mm. Hg." Brodie (1914) and Brodie and Mackenzie (1914) made careful measurements of the sizes of glomeruli and tubules before and during diuresis in cats and dogs, showing the marked distention of these structures during activity. Volhard (1935) states "The histological picture of kidney insufficiency, whether produced through subacute or severe chronic nephritis, through malignant sclerosis or amyloid disease, or through drastic reduction of healthy renal tissue, is always the same: an astonishing dilatation of the remaining tubules and a high grade flattening of their epithelial cells. . . . . a lesser degree of tubular dilatation and a slight flattening of the epithelium is observed as a transient phenomenon at the height of diuresis."

The third set of circumstances in which intrarenal pressure has been studied occurs in the isolated blood-perfused kidney of the dog. The matter arose from a study of the form of the ureter pressure, urine-flow curves (Winton, 1931 a) and of the venous pressure urine-flow curves (Winton, 1931 b), it being found that in most kidneys a rise in the obstructing pressure failed to affect the urine flow unless the rise exceeded a certain value, often about 10 mm. Hg. This was attributed (Winton, 1933) to an intrarenal pressure exerted laterally on some distal part of the tubules, keeping them collapsed until they were forced open by urine whch had reached a pressure just greater than that in the renal tissue. In support of this view it was shown that the urine secreted against different ureter pressures within the ineffective range had an unaltered composition; a change in the proportion of active glomeruli could, therefore, not account for the phenomenon. Acceptance of the view provided a simple method of measuring intrarenal pressure, in terms of the ureter pressure which must just be exceeded in order to produce a perceptible fall in urine flow. This value lies between 4 and 14 mm. Hg in most kidneys; in a typical kidney it is 10 mm.; beyond this value, the urine flow, ureter pressure curve is practically linear; since urine flow is abolished by a ureter pressure of 20 to 30 mm. it can be calculated that the urine flow in the unobstructed kidney would be 50 to 100 per cent higher if the intrarenal pressure could be removed. Even the small intrarenal pressure of the normally secreting kidney is, therefore, no negligible factor in determining the urine flow; but diuretics such as urea and sulphate increase it up to 20 or 30 mm. Hg. Cooling the blood perfusing the kidney to 8 or 10°C. raises the intrarenal pressure to about the same value (Winton, 1934 a), but, strangely, changes in arterial pressure within the range 80 to 180 mm. Hg produce much less, and may produce no detectable effect on the value of the intrarenal pressure (Winton, 1936). Measurements with a sclerometer of the changes in tenseness of kidneys have shown that this varies qualitatively with the intrarenal pressure. Decapsulation commonly about halves the intrarenal pressure.

Intrarenal pressure can be abolished in kidneys secreting at not too high a rate by placing the organ in an air-tight chamber—the arterial, venous and ureter cannulae passing through a cork to the outside—and lowering the pressure in the chamber below that of the atmosphere (Herman and Winton, 1936). If the extrarenal pressure be lowered in this way, by an amount about equal to the intrarenal pressure, measured in terms of the minimum effective ureter pressure as above, there

is a substantial diuresis which is completely reversible but cannot be increased by further lowering of the extrarenal pressure. The degree of the diuresis corresponds well with that predictable from the ureter pressure, urine flow curve. The way in which intrarenal pressure obstructs the escape of urine, mentioned above, is demonstrated further by the facts 1, that after lowering the extrarenal pressure, the smallest increase in ureter pressure reduces the urine flow, and 2, that after raising the ureter pressure beyond its minimum effective value, a lowering in the extrarenal pressure does not have its usual diuretic action.

In his lecture on *Experimental reduction of renal tissue* Verney (1929) gives an account of Rose Bradford's (1898, 1899) demonstration that removal of part of a kidney may result in an increase in urine flow, and of Pickford and Verney's (1929) demonstration that the reduction of renal substance by ligature of one of the two primary branches of the renal artery resulted in an immediate increase, often twofold or more, of the urine flow from the surviving half-kidney. The change in the composition of the urine accompanying the development of this polyuria of the reduced kidney was such as can be produced by an appropriate increase in arterial pressure; the polyuria was, therefore, due to increased glomerular filtration. Ligature of the branch reduced the blood flow, usually to about one-half; injection of a dye into the branch showed, by the limitation of staining to half the kidney, that there was practically no anastomotic connection between the vascular supplies of the two halves of this organ. Winton (1934 a) showed that the polyuria due to reducing the kidney was at least as striking in the organ perfused at 9°C. as at body temperature, and so confirmed that it was a glomerular rather than a tubular phenomenon. In a series of kidneys the intrarenal pressure was reduced on an average from 13 mm. to 9 mm. Hg by ligature of the branch at body temperature, and from 26 mm. to 13 mm. Hg, by similarly reducing the kidney at 9°C. Intrarenal pressure reduction is, therefore, the main factor producing this spectacular polyuria of the surviving half of a kidney when the arterial supply to the other half is arrested.

The question arises, does intrarenal pressure obstruct the outflow of blood, or only the outflow of urine? Evidence is conflicting. A rise in venous pressure, too small to reduce the urine flow, often increases it; this is accompanied by a reduction in blood flow, and presumably by an increase in glomerular pressure. These facts would be simply explained by supposing that intrarenal pressure was not exerting a pre-existing obstruction to the blood flow, but would be difficult to explain

if it were doing so. On the other hand, Pickford and Verney (1934) report 8 to 15 per cent increase in blood flow through one half kidney when the arterial branch to the other half is ligatured, and this, at the arterial pressure 100 to 120 mm. Hg employed is quite as much as one could expect from a reduction in intrarenal pressure; moreover, Herman and Winton (1936) observed an increase in blood flow due to lowering of the extrarenal pressure; perhaps the observations that decapsulation increases blood flow through the kidney in dogs (Hülse and Litzner, 1926; Stropeni, 1925) should also be interpreted in this sense. The matter is of considerable theoretical importance and requires further analysis.

Intrarenal pressure, then, plays an important part in determining the urine flow in the isolated kidney of the dog, in the freely secreting kidney in an anesthetized animal, and probably in disease involving acute swelling of the kidney in man. The question remains, does intrarenal pressure play a significant part in the normal kidney in the conscious animal? The answer cannot yet be given. Theobald and Verney's (1935 b) observation that even a small increase in ureter pressure reduces the urine flow cannot be certainly taken as evidence in this connection, because evidence of various kinds suggests the possibility that such an increase may produce a protective reflex lowering of the glomerular pressure; for example, the increase in urine flow produced by a small rise in ureter pressure in decerebrate dogs (Brodie and Cullis, 1906) is believed to be due to reflex stimulation of the kidney; in lightly anesthetized dogs, an increase in pressure in one ureter often produces no change in flow from the kidney concerned but an immediate reduction in output from the other kidney. Moreover Theobald and Verney's observations on the effects of intra-abdominal pressure suggest that mechanical disturbances may easily evoke reflex compensatory effects. Experiments are planned on the ureter pressure, urine flow relation in conscious dogs after denervation of the kidney; if one were to hazard a guess as to the result, it would be that intrarenal pressure plays no significant rôle at normal urine flows, but may perhaps act as one of the limiting factors at the height of diuresis.

The proportion of active glomeruli. The intermittent activity of glomeruli has been observed in frog's kidneys under conditions in which they secrete urine (Richards and Schmidt, 1924), and there seems no reason to doubt that it occurs under normal conditions. Evidence has been brought forward that a comparable variation in glomerular function occurs in the mammalian kidney; Khanolkar (1922) injected hemo-

globin, and Hayman and Starr (1925) injected Janus green B.; both excised the kidney, cut sections and estimated the proportion of glomeruli in which hemoglobin or the dye could be detected. Adrenaline decreased the proportion, caffeine increased it even up to 100 per cent. This evidence is much less direct than that in the frog, and whereas it seems likely that the proportion of active glomeruli can vary under favourable conditions, it seems uncertain as to whether this variation plays any significant rôle in circumstances in which actual secretion of the mammalian kidney has been studied. In the isolated kidney there is definite evidence against supposing that caffeine acts in this way, for Verney and Winton (1930) showed that the composition of the urine formed during caffeine diuresis was the same as that produced by a rise in arterial pressure; hence, if caffeine be supposed to increase the proportion of active glomeruli, so must a rise in arterial pressure increase this proportion; but this should manifest itself in a progressive reduction in the resistance to blood flow as the arterial pressure is raised. In fact, however, the resistance to blood flow increases with arterial pressure. The observations on adrenaline, mentioned in the section on the variation in glomerular pressure, would be difficult to reconcile with an explanation depending on variation in the number of active glomeruli. The remarkable parallelism between the maximum ureter pressure and the degree of diuresis, whatever the diuretic or antidiuretic agent, has been observed by Gottlieb and Magnus (1901), Henderson (1905) and many others. Brodie (1914), it is true, comments on the absence of such parallelism, though he gives no experimental evidence in support of this view, nor describes the condition under which this indeterminate relation can be reproduced. Cushny (1926) adopted Brodie's view, as conforming to the requirements of what he called the "modern theory." But there is no doubt that under ordinary experimental conditions the maximum ureter pressure is raised by most diuretic agents in anesthetized dogs and isolated kidneys. This might count as evidence against an increase in the number of active glomeruli being significant in the mechanism of diuresis. The enormous variation in urine, plasma concentration ratios of highly concentrated substances (e.g., 5–150 for inulin in the isolated kidney) with variation in urine flow in the mammalian kidney, contrasts with the relatively small change in these ratios in the frog, and may perhaps be taken as another hint that while the number of active units varies in the frog, it does not normally do so in the mammal. The only observation on the secretion of urine by the mammalian kidney, of which I

know, which may call for an explanation involving variation in the number of active glomeruli, occurs in the cold kidney; cold, like cyanide, appears to be unable to reduce the creatinine concentration ratio below about 1.5, and this ratio, unlike that in the warm kidney, seems to be unaffected by large changes in urine flow due to changes in arterial pressure (Bickford and Winton, 1937). The observation, however, requires confirmation before it is accepted.

Blood flow. The high blood flow through the kidney compared with that through most other organs is well-known. A table summarizing measurements on the dog's kidney published up to that time is given by van Slyke, Rhoads, Hiller and Alving (1934). The curious independence of the renal blood flow and the arterial pressure in the lightly anesthetized dog was shown by Rein and Rössler (1929) and confirmed by Glaser, Laszlo, Schürmeyer (1932, 1933) and by Unna (1935); this applies to fortuitous changes in arterial pressure, but particularly to the large changes which are brought about by carotid sinus reflexes. Unna, for example, found 50 to 80 mm. Hg rise in arterial pressure due to clamping both carotids, and a 30 to 50 mm. Hg fall in arterial pressure due to distention of one carotid sinus, had no detectable influence on the renal blood flow. Reduction in arterial pressure due to hemorrhage, however, reduces the blood flow through the kidney (Jannsen and Rein, 1928). Comparable observations on conscious dogs have not yet been published.

In the isolated kidney, increase in arterial pressure always produces an increase in blood flow, but the property that the resistance to blood flow increases with pressure appears to be unique to the kidney, other organs exhibiting a diminishing resistance with increasing pressure (Winton, 1932). This property is not due to increase in intrarenal pressure with arterial pressure, but it may be due to an increase in viscosity of blood flowing in the vasa efferentia due to a higher proportion of water being removed in the glomeruli at higher pressures. Such an increase in viscosity can be calculated from the measurements of the viscosity of blood of different corpuscular concentrations in the arterioles of the dog's hindlimb (Whittaker and Winton, 1933), and by allowing according to the Arrhenius law for the increase in concentration in the plasma proteins (Trevan, 1918). If the pressure-fall in the vasa efferentia be taken as about one-half of that from renal artery to vein, it can be calculated from the form of the blood pressure-flow curve that about 30 per cent of the water has been filtered off in the glomeruli at normal arterial pressures. This result is almost independent of the

degree of approximation involved in the subsidiary assumptions, since the increase in viscosity of the blood is so slow up to about a 30 per cent degree of concentration, and so surprisingly rapid thereafter. If the calculation is based on measurements of blood viscosity in glass tubes, the result depends on the viscometer; in relatively large bore tubes even with high velocity (e.g., Hess, 1912) types of viscometer the apparent viscosities are about double the values obtained in the hindlimb (Whittaker and Winton) or in glass tubes of about the same diameter as the arterioles (Bayliss, 1933); but the change of viscosity with change in concentration of blood is sufficiently similar, however measured, not to affect the result of this calculation appreciably. The creatinine clearance in a kidney, in which the filtration rate has been calculated from the blood pressure-flow curve, is rarely more than about one-third of this value of the filtration rate. This discrepancy will be considered in the section below on "Leakage from the tubules." Whether such a calculation gives the true filtration rate or not, it does, at least, define to some extent the limits which are set by a rise in viscosity of the blood flowing through the kidney.

The effect on the blood flow of applying pulsatile instead of steady arterial pressures in perfusion experiments has been studied by a number of authors, most of whom report that pulsation increases the flow, and may also increase the urine flow. These observations are open to two objections: 1. Many of them were performed on Ringer-perfused organs (e.g., Hooker, 1910; Hühne, 1916; Fleisch, 1919, 1920, and v. Uexküll, 1935), in which the intrarenal pressure is always the main factor obstructing the flow of the perfusion fluid; v. Uexküll, for example, reports Ringer flows in his dog's kidneys which are probably only 2 to 3 per cent of what the flow of the relatively viscid blood had been in the same organ at a similar arterial pressure. It is clear that such kidneys, and other edematous organs, will exhibit increased flow with pulsatile pressures in the same way as liquid just spilling over the open top of a vertical tube would increase its flow if the applied pressure became pulsatile, but this phenomenon has no relevance to normal conditions in which the resistance to flow is due to shear within the blood vessels and not to pressure outside the vessels tending to obliterate them. 2. In these experiments, the aim has been to make the mean pulsatile pressure equal to the steady pressure with which it is compared. But presumably the R.M.S. value of the pressure should be chosen for a fair comparison, and this with the pulse wave-forms used may be widely different from the mean. Hooker (1910), in fact, reports that the

greater the amplitude of his pulsations the greater the flow even through a tube constricted by a screw clamp! A number of observers who have made preliminary observations on the heart-lung-kidney hold rather conflicting opinions about the effects of pulsation on both urine and blood flows; lack of agreement may be due to the fact that the effects are small, and may vanish when the intrarenal pressure is relatively low.

Leakage from the tubules. Low threshold diuretics, such as urea, sulphate and sucrose, increase the maximum ureter pressure in isolated kidneys and in anesthetized dogs from the normal "resting" value of about 30 mm. Hg to about 70. mm Hg. The same substances lower the minimum arterial pressure consistent with urine formation from about 75 mm. to about 40 mm. Hg. These facts are not easy to interpret on current views of renal secretion; for, according to the filtration reabsorption hypothesis, when the ureter pressure is high enough to stop urine flow, reabsorption of water from the tubule continues until the osmotic pressure of the non-reabsorbed substances is high enough to stop it. If there exists any substance which is not reabsorbed, reabsorption of water must cease; because, if it continued, the tubules would become blocked with a solid deposit of the non-reabsorbed substance. If reabsorption ceases, and there is no urine flow, glomerular filtration must also cease, and the whole of the contents of the tubule should be stationary, both physically and chemically. Hence the maximum ureter pressure should be equal to the glomerular capillary pressure minus the colloid osmotic pressure of the plasma proteins. The "resting" maximum ureter pressure of 30 mm. is, therefore, lower than it should be, to fit in with other consequences of the filtration-reabsorption hypothesis. The fact that urea increases it, is in direct conflict with the evidence that this substance exerts its diuretic effect by action on the tubules. An exactly similar argument can be stated about the minimum arterial pressure. Similar effects on both maximum ureter pressure and on the minimum arterial pressure are produced by cyanide (Starling and Verney, 1923) and by cooling (Bickford and Winton, 1937), the former increasing the blood flow by about 30 per cent (Bayliss and Lundsgaard, 1932), and the latter about halving the blood flow. It is particularly difficult to suppose that these raise the glomerular pressure, since they reduce the urea, creatinine and inulin clearances to about one quarter at normal arterial pressures.

The simplest escape from these difficulties, compatible with the filtration-reabsorption hypothesis in its original, or in its modern form

including secretion of some substances into the tubules, is to suppose that when flow from the ureter ceases, urine flow down the tubules still takes place, this urine leaking back into the blood at some point distal to the site of reabsorption of water. The flow of this urine involves a pressure fall along the tubules, and hence explains the low maximum ureter pressure and the high minimum effective arterial pressure in the kidney secreting at normal rates; it explains the action of urea, etc., in raising the former and lowering the latter of these pressures when secretion is at a high rate. If this be so, it can be calculated from the form of the urine flow, ureter pressure curves in isolated kidneys, that two or three times as much urine may leak away as emerges from the ureter at normal rates of flow, the proportion leaking becoming smaller the higher the urine flow (Winton, 1934 b). A leakage of the same order of magnitude has, as indicated above, been calculated from the form of the blood pressure-flow curve of the isolated kidney. As far as the rather indefinite evidence available goes, it seems likely that the creatinine and inulin clearances in isolated kidneys are only one-half to one-third of the values obtained in conscious dogs. The creatinine clearance, which is identical with the inulin clearance in conscious dogs (Shannon, 1935 a, b), is less than the inulin clearance at low rates of urine flow in isolated kidneys (Shannon and Winton, 1937).

Evidence derived from the maximum ureter pressure, the minimum effective arterial pressure, the blood pressure-flow curve, the absolute value of clearances, and from the ratio of inulin to creatinine clearances, all points, therefore, to the existence of a substantial leakage from the distal part of the tubules in the isolated kidney of the dog. The first two items of evidence are in favour of a leakage also occurring in anesthetized dogs, the last two items are, if anything, against such a leakage in anesthetized dogs. Hinman and Lee Brown (1924) hold that leakage from the Fornix calycis has a physiological significance, and this view is developed in connection with experimental work on hydronephrosis by Fuchs (1932, 1933, 1934, 1936). It seems, however, that the pyelovenous back-flow contemplated by these authors may be different from the leakage in the isolated kidney, for in the latter no applied ureter pressure will drive liquid from the pelvis into the blood, unless the pressure is great enough to rupture the tissues.

Bayliss and Lundsgaard (1933) attribute the reduction in creatinine clearance in the isolated kidney due to cyanide to the development of a leakage in the glomerular capsule itself, or at least in some very proximal part of the tubule; this view is based, in part, on Richards' (1929)

demonstration that cyanide increases the permeability of the tubules in the frog's kidney. This view does not account very simply for the increase in maximum ureter pressure produced by cyanide, but it can, perhaps, be made to do so by allowing for variation in the co-existing leak from the distal part of the tubules.

It is evident that a leak from the tubules, back into the blood, plays an important part in kidneys under certain conditions. It is possible that it plays no part in the normal kidneys in conscious animals, but it is not unlikely that it will appear in disease.

The pressure-fall between glomerular capillaries and ureter. The total pressure-head available for driving urine down the tubules must suffice to include three pressures in series: 1, the colloid osmotic pressure of the plasma proteins; 2, the pressure-fall across the glomerular membrane, and 3, the pressure-fall along the tubules. 1. The action of colloid osmotic pressure in impeding glomerular filtration (Starling, 1899) is generally accepted. The character of the filtration process has recently been given greater precision by Bayliss, Kerridge and Russel (1933) who showed that proteins with a molecular weight exceeding 67,000 were retained; those with a lower molecular weight were transmitted by the glomerular membrane. The diuretic action of dilution of the blood with saline, however, which has in the past been used as evidence in favour of this conception, presents some difficulties; for the diuresis is quite disproportionately great compared with the effects of a change in arterial pressure which would produce a rise in glomerular pressure comparable with the fall in colloid osmotic pressure due to the dilution with saline. The disproportion is over tenfold. The probable explanation is that the main action of saline is on the tubules, and hence its relatively inconsiderable action on cold kidneys. The colloid osmotic pressure in dog's blood is now taken as 20 to 25 mm. Hg, and this would be the effective value in the glomeruli if there were no filtration; during normal activity, however, the osmotic pressure which can be overcome by the hydrostatic pressure available is the colloid osmotic pressure of the most concentrated blood as it leaves the glomerular capillaries; this may be taken as about 40 mm. Hg, if the current methods of estimating glomerular filtration rates and blood flows through the kidney are valid.

2. Krogh (1929) and Rehburg (1935) have calculated the pressure needed to drive the glomerular filtrate, measured in terms of the creatinine clearance, across the glomerular membrane in man. Taking Vimtrup's (1928) figures for the area of the membrane (1.5 sq.m. for

both kidneys), Rehberg's values for glomerular filtration (180 cc. per min.) and Landis' (1927) measurements on the pressure needed to drive liquid across capillaries in the frog's mesentery, it appears that something of the order of 60 mm. Hg hydrostatic pressure difference across the glomerular membrane would be needed if the permeability of the membrane were the same as that of the capillaries.

3. The pressure-head necessary to drive liquid down the tubules has not yet been measured, and calculations based on the Poisseuille Law and on measurements of the sizes of the tubules are difficult, partly because the tubules are elastic and leaky, partly because the measurements in microscopic sections may not correspond with the normal values. Nevertheless Brodie (1914) calculated on the basis of measurements on dog's kidneys that a pressure-head of the order of 80 mm. Hg would be needed to drive the liquid from the intracapsular space to the ureter, even supposing that the glomerular filtrate was formed at only the same rate as the urine, i.e., that there was no reabsorption of water. O'Connor (1924) from similar estimates on rabbit's kidneys concluded that at least 25 mm. Hg would be needed to account for the pressure-fall along the tubules.

Putting these pressures together, we have 40 mm. for the colloid osmotic pressure, 60 mm. filtration pressure, and 25 to 80 mm. pressure-fall along the tubules,[1] i.e., a total of 125 to 180 mm. Hg. A kidney will secrete in this way at an arterial pressure of 120 mm. involving a glomerular capillary pressure probably of about 80 mm. Hg. It is clear that the experimental evidence, underlying the pressure-flow relations which are implicit in the filtration-reabsorption theory, is far from satisfactory, and as long as this remains so, renal physiology can hardly be regarded as secure from an intrusion by the unexpected.

REFERENCES

BAINBRIDGE, F. A. AND C. L. EVANS. J. Physiol. **48**: 278, 1914.
BAYLISS, L. E. Cit. by WHITTAKER AND WINTON, 1933.
BAYLISS, L. E. AND E. LUNDSGAARD. J. Physiol. **74**: 281, 1932.
BERGLUND, H. AND J. MEDES. The kidney in health and disease. London, 1935.
BICKFORD, R. J. AND F. R. WINTON. J. Physiol. **78**: Proc. 15, 1933.
BICKFORD, R. J. and F. R. WINTON. J. Physiol. **89**: 198, 1937.
BRADFORD, J. R. Lancet **1**: 765, 1898; J. Physiol **23**: 415, 1899.
BRODIE, T. G. Proc. Roy. Soc. **87B**: 571, 1914.

[1] The hypothesis that water is reabsorbed implies a considerably greater estimate of the pressure fall along the tubules. A factor for intrarenal pressure may have to be added to this.

BRODIE, T. G. AND W. C. CULLIS. J. Physiol. **34**: 224, 1906.
BRODIE, T. G. AND J. J. MACKENZIE. Proc. Roy. Soc. **87B**: 593, 1914.
CANNY, A. J., E. B. VERNEY AND F. R. WINTON. J. Physiol. **68**: 333, 1930.
CONWAY, E. J. J. Physiol. **60**: 30, 1925.
CUSHNY, A. R. J. Physiol. **28**: 431, 1902.
The secretion of the urine. 1st ed. London, 1917. The secretion of the urine. 2nd ed., London, 1926.
DREYER, N. B. AND E. B. VERNEY. J. Physiol. **57**: 451, 1923.
DUNGERN, M. Ztschr. Exper. Med. **97**: 110, 1935.
EBBECKE, U. AND E. H. LEACH. Pflüger's Arch. **233**: 419, 1933.
EKEHORN, G. On the principles of renal function. Stockholm, 1931.
ELLINGER, P. Handb. d. Norm. u. Path. Physiol. **4**: 308, 1929.
EPPINGER. Therap. Halbmonat. **35**: 225, 1921.
FLEISCH, A. Pflüger's Arch. **174**: 177, 1919.
Pflüger's Arch. **178**: 3, 1920.
FUCHS, F. Ztschr. Urol. Chirug. **33**: 1, 1931.
Ztschr. Urol. Chir. **37**: 154, 1933. Urologia N2, 1934; Ztschr. Urol. Chir. **42**: 80, 1936.
FUCHS, F. AND G. FUCHS. Ztschr. Exper. Med. **81**: 784, 1932.
GLASER, H., D. LASZLO AND A. SCHÜRMEYER. Arch. Exper. Path. und Pharmakol. **167**: 292, 1932; Arch. Exper. Path. und Pharmakol. **168**: 139, 175, 1933.
GOTTLIEB, R. AND R. MAGNUS. Arch. f. Exper. Path. **45**: 223, 1901.
GRIFFITH, J. Q. AND H. R. HANSEL. Am. J. Physiol. **74**: 16, 1925.
GRÜTZNER, P. Pflüger's Arch. **11**: 372, 1875.
HARRISON, R. Lancet **2**: 1080, 1896.
HAYMAN, J. M., JR. Am. J. Physiol. **79**: 389, 1927.
J. Clin. Invest. **8**: 89, 1929.
HAYMAN, J. M., JR. AND I. STARR, JR. J. Exper. Med. **42**: 641, 1925.
HEIDENHAIN, R. Hermann's Handb. d. Physiol. **5**: (i), 1883.
HEMINGWAY, A. J. Physiol. **71**: 201, 1931.
HENDERSON, V. E. J. Physiol. **33**: 175, 1905.
HERMANN, M. Sitzber. Akad. Wiss. Wien. Physic. Math. **36**: 349, 1859.
HERMAN, F. G. AND F. R. WINTON. J. Physiol. **87**: 77 Proc., 1936.
HESS, W. R. Münch. Med. Wchnschr. **32**: 1590, 1907; Arch. Anat. Physiol. Leipzig, p. 197, 1912.
HINMAN, F. AND R. K. LEE BROWN. J. A. M. A. **82**: 607, 1926.
HOOKER, D. R. Am. J. Physiol. **27**: 24, 1910.
HÜHNE, H. Pflüger's Arch. **165**: 180, 1916.
HÜLSE, W. AND S. LITZNER. Ztschr. Exper. Med. **52**: 84, 1926.
JACOBJ, C. Arch. f. Exper. Path und Pharmakol. **136**: 203, 1928.
JANNSEN, S. AND H. REIN. Ber. Physiol. **42**: 567, 1928.
JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH. Am. J. Physiol. **100**: 301, 1932.
KHANOLKAR, V. R. J. Path. and Bact. **25**: 414, 1922.
KLISIECKI, A., M. PICKFORD, P. ROTHSCHILD AND E. B. VERNEY. J. Physiol. **72**: 26 Proc., 1931.
KROGH, A. The anatomy and physiology of capillaries. New Haven, 2nd ed., p. 348, 1929.

LANDIS, E. M. Am. J. Physiol. **82:** 217, 1927.
LIVINGSTON, A. E. J. Pharmacol. Exper. Therap. **32:** 181, 1928.
LOEBELL, C. F. Cit. from ELLINGER, 1929.
LUDWIG, C. Lehrbuch d. Physiol. 2nd ed., 1861.
MARSHALL, E. K., JR. AND M. M. CRANE. Am. J. Physiol. **64:** 387, 1923.
MARSHALL, E. K., JR. AND A. C. KOLLS. Am. J. Physiol. **49:** 302, 1919.
MAYRS, E. B. AND J. M. WATT. J. Physiol. **56:** 120, 1922.
MENDENHALL, W. L., E. M. TAYLOR AND A. N. RICHARDS. Am. J. Physiol. **71:** 174, 1924.
NEWTON, W. H. Recent advances in physiology. London, 1936.
O'CONNOR, J. M. J. Physiol. **59:** 200, 1924.
OZORIO DE ALMEIDA, A. C. R. Soc. Biol. Paris **96:** 385, 1927.
PICKFORD, M. AND E. B. VERNEY. Am. J. Physiol. **90:** 470, 1929.
J. Physiol. **82:** 16 Proc., 1934.
REHBERG, P. B. Biochem. J. **20:** 447, 1926.
In BERGLUND and MEDES. Kidney in health and disease. London, p. 75, 1935.
REIN, H. AND R. RÖSSLER. Ztschr. Biol. **89:** 237, 1929.
RICHARDS, A. N. Methods and results of direct investigation of the function of the kidney. Baltimore, 1919.
In H. BERGLUND and J. MEDES. The kidney in health and disease. p. 19. London, 1935.
RICHARDS, A. N. AND O. H. PLANT. Am. J. Physiol. **59:** 144, 1922a; 1922b. Ibid. **59:** 184; Ibid. **59:** 191, 1922c.
RICHARDS, A. N. AND C. F. SCHMIDT. Am. J. Physiol. **71:** 178, 1924.
RHOADS, C. F., D. D. VAN SLYKE, A. HILLER AND A. ALVING. Am. J. Physiol. **110:** 392, 1934.
SHANNON, J. A. Am. J. Physiol. **112:** 405, 1935a; Ibid. **114:** 362, 1935b.
SHANNON, J. A. AND F. R. WINTON. (In preparation for publication.)
SLYKE, D. D. VAN., C. P. RHOADS, A. HILLER AND A. ALVING. Am. J. Physiol. **109:** 336, 1934.
SOLLMANN, T. Am. J. Physiol. **10:** xxv, 1903; Am. J. Physiol. **13:** 241, 1905.
STARLING, E. H. J. Physiol. **19:** 312, 1896.
J. Physiol. **24:** 317, 1899.
Principles of human physiology. London, 1st ed., p. 1277, 1912.
STARLING, E. H. AND E. B. VERNEY. Proc. Roy. Soc. **97B:** 321, 1925.
STROPENI, L. Arch. Ital di Chir. **13:** 472, 1925.
TAMMANN, C. Ztschr. Physik. Chem. **20:** 180, 1896.
THEOBALD, G. W. AND E. B. VERNEY. J. Physiol. **83:** 341, 1935a.
Quart. J. Exper. Physiol. **25:** 341, 1935b.
THORINGTON, J. M. AND C. F. SCHMIDT. Am. J. Med. Sci. **165:** 880, 1923.
TREVAN, J. W. Biochem. J. **12:** 60, 1918.
UEXKÜLL, T. v. Pflüger's Arch. **235:** 534, 1934.
UNNA, K. Pflüger's Arch. **235:** 515, 1935.
USTIMOVITSCH, C. Ber. Sächs. Ges. Wiss. **12:** 430, 1870.
VERNEY, E. B. Lancet **216** (i): 643, 1929.

VERNEY, E. B. AND E. H. STARLING. J. Physiol. **56**: 353, 1922.
VERNEY, E. B. AND F. R. WINTON. J. Physiol. **69**: 153, 1930.
VIMTRUP, B. Am. J. Anat. **41**: 123, 1928.
VOLHARD, F. In BERGLUND and MEDES. Kidney in health and disease. London, p. 249, 1935.
WHITTAKER, S. R. F. AND F. R. WINTON. J. Physiol. **78**: 339, 1933.
WINTON, F. R. J. Physiol. **71**: 382, 1931a; J. Physiol. **72**: 49, 1931b; J. Physiol. **72**: 361, 1931c; J. Physiol. **78**: 151, 1931d; Trans. XIVth Congresso Internaz. di Fisiol. p. 264, 1932; J. Physiol. **78**: 9 Proc., 1933; J. Physiol. **82**: 27 Proc., 1934a; J. Physiol. **83**: 38 Proc., 1934b; J. Physiol. **87**: 18 Proc., 1936.

FATIGUE IN MENTAL WORK

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Since the meaning and justification of all three terms in the title of this paper have been subjects for a great deal of controversy, and since the interpretation of the experimental work in this field is impossible unless some common basis of understanding is first arrived at, I propose to begin with a somewhat arbitrary definition of terms.

Mental work. Both of these words are highly ambiguous. What is *work*? If we take over the usage of physics and mechanics, work is reducible to a simple formula. The equation for physical work, analogous to that for physical energy, would be $\frac{1}{2}MV^2$ where M is mass moved and V is distance traversed divided by time consumed. Physiologically, work is consumption of energy, and rate of work is computed in terms of rate of energy consumption. The work of a living organism, then, would be measured by the increase in the metabolic rate of the working over that of the resting organism. If there is no increase in metabolic rate, then, by definition no work has been performed. The fallacy in this whole point of view is the assumption that there must be a constant ratio between *energy expenditure* (as measured by calories consumed, for example) and *output*. Actually these two variables are not well correlated. The caloric energy expended by the organism is only remotely related to its accomplishment. Therefore, the term "work" must be identified with either one or the other, but certainly not with *both* these variables. From the psychologist's viewpoint, it is more profitable to identify "work" with the *product*, the *output*, which is readily measurable and objective.

The other term, *mental*, is equally ambiguous. If we immediately set aside all antiquated metaphysical dogmas about a unique "psychic" process, and accept a whole-hearted monism which identifies mental activity with the sensory-neuro-muscular processes of the organism, i.e., behavior, nevertheless we are still confronted with the problem of distinguishing "mental work" from "muscular work". When is an individual performing exclusively "*mental* work"? Several attempts

have been made to differentiate. Thorndike suggested that "mental work" should refer to work of the "connecting system", i.e., the nervous system, as opposed to work of sense organs and muscles. But all of the experimental work on the activities in muscles and glands during thinking shows that such a distinction is highly artificial, and falls down practically, because no task can be devised in which there is no component of muscular tensions such as are involved even in the mere act of attending to a stimulus (15). Watson distinguished between two kinds of behavior—overt and implicit, the former referring to the observable responses of the larger skeletal muscles in acts directed toward the environment, the latter referring to the obscure sensory-neuro-muscular-glandular adjustments inside the organism which occur in mental activities like perceiving, reasoning, emotion, etc. To him, mental work meant largely "implicit behavior" involving, to be sure, some finer muscular processes, but little or no gross muscular effort. I shall arbitrarily accept this definition. Thus lifting weights is "muscular work" even though it does involve the nervous system, and a task like doing arithmetical problems is "mental work" even though sensitive instruments show that it involves muscular contractions. Just to illustrate the necessity of a clear understanding on these points, let me refer to a recent report on metabolism in mental work by Benedict and Benedict (3). Studying the change in metabolic rate from performing problems in addition and in multiplication without paper or pencil as compared with an alert resting state, they came to the following conclusion:

> The increase in heat production as a result of intense mental effort of this type can hardly be of the order of more than three or four per cent. These small increases in oxygen consumption and heat production are in large part to be accounted for by the increased muscular activity accompanying the increased ventilation of the lungs and the increased heart rate. Hence, making a most conservatively small allowance for the effect of increased circulatory and respiratory activities in this small metabolic increase, we conclude that mental effort *per se* is without significant influence upon the energy metabolism.

Now it is perfectly obvious that these authors are thinking of "mental effort" as exclusively activity of a limited part of the nervous system; and hence, they must exclude from the results even the metabolic effect of the increased circulatory and respiratory activities which are an indispensable product of the nervous activity. The wonder is that these authors, and others who came to similar conclusions, (because they started with similar premises) ever performed the studies in the first place, since it is obvious, a priori, that the increased activity of a limited

number of brain cells could not add any appreciable amount to the metabolic rate of the total body.

But from the point of view of the definition of "mental work" adopted in this paper, Benedict and Benedict are not justified in excluding the increased muscular activity involved in circulatory and respiratory effort from the "mental work", or "mental effort." The conclusion to be drawn from their excellent study would be that mental effort produces a small but significant increase in metabolic rate.

Fatigue. This term, as commonly employed, is, if possible, even more ambiguous than the terms *work* and *mental.* It can refer to at least three entirely different, and, to a certain extent, independent things. By derivation it means "weariness" or "feelings of exhaustion"; in other words, the subjective experience of the worker. But as used by the physiologist, it usually means that change in the condition of the cells or organs which have undergone excessive activity resulting in a loss of power. And from the point of view of output or product, it means that decrement in the quantity or quality of the product which results from the continuity of the work. We might call these 1, the subjective; 2, the organic, and 3, the product definitions of fatigue. If experiment had shown that they were closely correlated, then we could dispense with the triple problem, but all results point to their relative independence of one another. Some authors, feeling that the organic change is the most fundamental, identify it with the term fatigue, and make the other two variables concomitants of it. This is impractical; because, in fatigue studies, large variations in subjective weariness or in output can appear in the absence of any comparable organic change. Expediency dictates the separate consideration of all three variables, though *product* is the one most often measured because it is most accessible and most objective. Some authors recommend the complete abandonment of the term fatigue, due to its ambiguity (28). The reviewer prefers to use it as a generic term to include all three variables.

To summarize, then, "fatigue in mental work" means a measurable decrement in either output or organic state or subjective feeling-tone resulting from the continuous performance of intellectual work involving implicit behavior predominantly.

Measurement of fatigue. Each of the three different aspects of fatigue requires its own measuring device and criteria. Subjective fatigue is measured by the change in feeling-tone described as loss of readiness for work, or increasing desire to terminate work. Like all subjective variables it must be measured by means of a rating scale based on the

worker's introspective report. The simpler such a rating scale is the better. For example, the worker may be asked to designate his feeling-tone at the beginning and at regular intervals during work on a scale of five degrees; 1, very fit; 2, fit; 3, average; 4, rather tired; 5, very tired. From these values a curve can be plotted whose horizontal axis represents successive equal units of continuous work or time, and whose vertical axis represents the levels of feeling-tone. The curve would then indicate the progressive deterioration in feeling-tone with continued work.

Organic fatigue, on the other hand, is measured in terms of quantitative change in metabolism. Many measures of metabolism are used, which can be roughly divided into 1, changes in the character of physiological processes like breathing, heart rate, blood pressure, blood volume, temperature, etc., and 2, changes in the character of the products of metabolism, as carbon dioxide elimination in the expired air, oxygen consumed, the respiratory quotient, blood analysis and urine analysis for inorganic phosphorus, lactic acid, alkali reserve, hemoglobin, decreased O_2 or increased CO_2, decreased sugar, changes in calcium, chlorides, cholesterol and creatinine contents. Some authors have attempted to study the metabolism of the brain exclusively by obtaining their blood samples from internal jugular vein punctures or by a study of cerebral thermometry.

Objective fatigue, or decrement in output, is studied by one or other of two main methods, the continuous work method and the interpolated task method. According to the former, a continuous record is kept of the output of the subject per unit time throughout the period of work. A work curve is then plotted from these values showing the change in level of performance of the subject in successive units of time. The criteria of efficiency of performance most commonly used are speed and either accuracy or some other criterion of quality of output. According to the other, the interpolated task method, the subject is given a brief test at stated intervals during the work period in which his level of performance is tested on some other task than the one on which he is engaged. For example, if the main task is addition of numbers, the interpolated test may be of strength of grip on a dynamometer, or physical endurance on an ergograph or of eye-hand coördination, or of visual reaction time, or sensory acuity. It is assumed that the interpolated test is a better or more direct test of fatigue than the work task itself, which is, of course, a highly questionable assumption. Most recent investigators prefer the continuous work method, unless they

are investigating progressive changes in efficiency resulting from other factors than continuous work, such as insomnia, fasting, drugs, diet, etc. In measuring fatigue by the continuous work method, the resulting trend of the curve of work is complicated by certain factors which make it unsafe to estimate the degree of fatigue entirely from the decrement in the curve itself. One of these factors is practice effect. Unless the subject is highly practiced in the task, his performance will improve considerably in a single work period, thereby masking the fatigue effect and giving either a level or rising curve with no apparent decrement. To obviate this difficulty, Thorndike (40) suggested that, since fatigue is defined as a lowered capacity for work which is removed by rest, it is legitimate to compare a subject's level of performance at the end of a work period with his performance level as tested after a subsequent rest of sufficient length to remove the effect of the work. By subtracting the former from the latter, one can obtain the true fatigue decrement.

Other disturbing factors are due to sudden changes in the subject's motivation, causing his performance level to change temporarily. For example, "end spurt" is the abnormally high point of performance near the very end of a work period caused by the subject's elation over the approaching rest. It is hard to say *when* a subject is working at maximum capacity and when not because of these changes in the strength of the motive to which he is responding. We have to assume that, if he is instructed to work at maximum capacity at all times, then his level of performance at any given time does represent his maximum at that particular time.

One further question needs to be clarified before the results of experimental work can be reviewed; that is the question of choice of mental-work tasks. If results are to be treated quantitatively, tasks must be used which are divisible into equivalent units as to amount and difficulty. Sometimes it is desired to compare fatigue from tasks of different degrees of difficulty. But what is the criterion of *difficulty* of a mental task? Some authors judge by the subject's own estimate. If the subject feels that the performance of the task is extremely arduous, then it is judged to be a difficult task. For example, Benedict and Benedict make this the main criterion in selecting "mental multiplication" of two-place by two-place numbers. But what justification is there for this in the light of the known fact that a greater decrement can be obtained from twenty minutes of color-naming than from two hours of "mental multiplication"? Subjective difficulty is no criterion of objective

difficulty. As will be shown later, Robinson and Bills (32) found *homogeneity* to be the most important single factor productive of rapid decrement in mental work, and hence, the most important criterion of difficulty on the objective side. It is evident that difficulty must be defined differently depending on whether subjective or objective fatigue is being investigated.

RESULTS OF EXPERIMENTAL STUDIES OF MENTAL FATIGUE. The following review of experimental work is admittedly incomplete. The limitations of space have necessitated confining the references to a few typical studies of recent date. The aim is to indicate present trends rather than to give a complete historical account.

A. *Feeling-tone.* All investigators are agreed in finding a much more rapid decrement in feeling-tone than in objective output of mental workers. A typical study is that of Poffenberger (30) on the decrement developing in four tasks of varying homogeneity; addition, sentence completion, intelligence tests, and judging compositions. He took introspective reports of the subject's feeling-tone after each twenty-one minute period during five and a half hours' work. Seven degrees of feeling-tone were recorded from "extremely good" to "extremely tired". Output and feelings were plotted separately. The results show that feeling-tone dropped from the very start and continued to decline, whereas output remained at a constant level in two of the tasks, dropped in one, and increased in the fourth, due probably to practice. The persons showing greatest feeling-tone decrement also showed greatest output decrement, but, otherwise, the two processes were unrelated. This is in agreement with previous findings. Persons differ considerably in their susceptibility to feeling-tone decrement from repetitive work. It is difficult to make direct comparisons because one man might rate himself "extremely tired" when he actually felt no worse than another man who gave himself a rating of "moderately tired." But, in general, the more intelligent, more versatile, and less perseverative type of thinker is most quickly bored, and hence, has the greatest feeling-tone decrement.

B. *Organic state.* The effect of mental work, as here defined, on the organic condition of the worker can be stated, but only with reservations, due to the wide discrepancy between results of different studies. Part of this discrepancy is due to the different ways in which the term "mental work" was interpreted by the different investigators. Some of them were obviously searching for a "will-o-the-wisp." Let us first

do a little a priori speculating on the question of what the physiological effects of mental work, as defined by us, might be expected to be. Then we can determine whether the actual results of experiment bear us out.

Mental work has been defined as the implicit behavior involved in solving intellectual problems. What physiological mechanisms does this implicit behavior use, and what fatigue effects should be anticipated? Roughly, these mechanisms are sense organs, peripheral and central nerves, striped and smooth muscles and glands. That a certain amount of sense organ fatigue can be expected, is evident from the use of the sense organs in receiving the stimuli, and such fatigue manifests itself objectively in decreased sensory acuity. Fatigue of the nervous system was once denied but can no longer be, in the face of the evidence showing, on the one hand, that there is increased metabolism in nervous tissue when impulses are conducted (16), involving production of CO_2, dissipation of heat, etc.; and, on the other hand, that continuous stimulation of nerve is followed by a decreased capacity to conduct which develops cumulatively (14). This is true independently of the phenomenon of refractory phase. That muscular fatigue is involved requires proof, and there is abundant experimental proof at hand. Studies of the skeletal muscles show increased tension accompanying all thought processes (10, 15, 24), and proof is not lacking that this muscular tension is not merely an irrelevant concomitant but is an essential element in stimulating and directing the thought process (4). The process of *attention*, the term which is used for a state of mental alertness, is also a process of *tension*. Smooth muscles and glands are also involved. Studies of blood pressure, heart rate, and breathing demonstrate smooth muscle involvement, while studies of altered electrical conductivity of the skin, the galvanic skin response, have shown it to be intimately related to the intensity of mental effort (37). We could predict this, from the evident fact that no intellectual response is completely devoid of emotional components; the total organism is too intimately associated to permit such an artificial process as a "purely cognitive" response to occur. And, where emotion is involved, smooth muscle and gland are affected.

Finally, there is a modicum of overt response involved in all practical mental work situations, even though it is rightly held at a minimum. This is not to be considered some foreign factor, to be subtracted from the mental work, but is an intrinsic aspect of it. All mental work, as it actually occurs in practical life, involves this overt element. Such a

highly artificial situation, as would be required to eliminate it, would have no practical predictive value for actual life.

We are now in a position to evaluate the results of the studies of energy expenditure in mental work.

1. *Changes in physiological activities.* a. Breathing rate and depth are both affected. A small increase in rate is accompanied by a great change in the quality of the breathing curve. It becomes very irregular with stretches of shallow breathing interspersed with deep inhalations (3, p. 36). In concentrated attention, the worker tends to hold his breath. To the extent that thinking involves sub-vocal speech, breathing is made irregular (19).

b. Pulse or heart rate has been found to increase slightly in those studies where the experimenter was careful to achieve a completely resting pulse before mental work was begun (13). Early studies by Vaschide and des Bancels, in which they compared their pulse rate on days when they rested and days when they did mental work and got a faster pulse on the rest days, are invalidated, because they failed to control their physical activity on the rest days. Arai's (1) results showing a drop in pulse rate of 2.3 per cent during a day's mental work are invalidated because she may have been physically active when the work began and during the work was physically passive. Dodge reports that "within the limitation of my experimental data, I can say that, provided one starts from a pre-experimental condition of relatively complete physical and mental relaxation, every new mental or physical activity is correlated with an accelerated pulse." Benedict and Benedict (3) recently obtained increases in heart rate from a resting rate of 60 to a rate of 65 per minute during an hour's mental work.

c. Temperature changes give contradictory results. All experimenters report that some subjects show a rise, while others show a drop, the amount never exceeding a half degree either way.

d. Blood pressure and volume have rarely been studied in this connection, but Day (11) reports no consistent tendency. Gillespie, on the contrary, obtained increases in blood pressure (17). Emotional stimuli are known to cause decided increases, as in the case of students facing an examination.

2. *Studies of the products of metabolism.* Since all these physiological activities can vary in the absence of any change in fundamental metabolism, it is necessary to study directly the products of the metabolic process.

a. *Respiratory calorimetry* is studied through an analysis of the gaseous exchange. Determinations are made of the oxygen intake, rate of oxygen consumption, and carbon dioxide elimination, through chemical analysis of the expired air. The respiratory quotient is the ratio between the oxygen absorbed and the carbon dioxide exhaled, indicating the economy of the combustion process. Probably the best single index is the oxygen consumption rate, because the carbon dioxide elimination rate varies with strength of breathing, and may not indicate any change in rate of carbon dioxide production at the time. When a person's resting metabolic rate is known, one can compute the percentage increase over this, caused by work. Muscular work has usually been found to give large increases, roughly proportional to the intensity of muscular effort, but not cumulatively proportional to the length of time over which the work continues. An oxygen debt is incurred which is paid back after the work ceases.

But for *mental* work, the results obtained by several investigators seem, on the surface, to be contradictory. Numerous recent studies report increases all the way from zero up to 28 per cent and more, above the resting rate (3) (34). A careful examination of the reports, however, suggests that the differences are due to differences in type and amount of work, in procedure, and in interpretation. The mental work used by those who got the largest increases involved more gross muscular activity. Probably, for tasks in which the muscular component is at an absolute minimum, an increase of 3 per cent to 4 per cent is a good conservative estimate. The effect appears to be non-cumulative; that is, the adjustment to the higher rate occurs early and remains at this level during the work period. A recovery period then follows, in which the return to normal is gradual. A good review of recent literature can be found in Benedict and Benedict (3).

b. *Blood analyses* have been made for the organism as a whole, and for the brain alone by internal jugular vein and carotid artery punctures (29). More CO_2 and less O_2 and less sugar have been found in blood leaving the brain than in blood entering, showing that the brain undergoes metabolism, but tests of outgoing blood before and after mental work show an increased O_2 and decreased CO_2 content (26). This is readily explained as due to the known dilatation of the vessels in the brain by mental effort, causing a greater volume of blood to pass through. Blood changes in the whole organism, from mental work, are small as might be expected. Decreased sugar has been noted, small increases in inorganic phosphorus, cholesterol, and creatinine, and a

lowered alkali reserve (18, 23, 25). Goldstein reports that when the muscular component is largely removed from the mental work, the blood changes from an hour and a half of work show no reliable difference from those resulting from an equal period of rest. He found no variations resulting from varying the intensity of effort.

c. *Urine analyses* indicate a small increase in organic phosphorus after prolonged mental work (39).

d. *Saliva tests* have been used occasionally with contradictory results, but the variations of hydrogen-ion concentration of the saliva do not correlate highly with those of the blood, and doubt has been raised as to the reliability of such determinations (2).

It would seem that the experimental demonstration of energy expenditure in mental work is already sufficient. What we need now is to determine what conditions of work require more or less energy expenditure. Noise is an example (22). Adequate or inadequate motivation is another.

It would also seem to be a fruitful direction for future research to determine whether the organic effects of mental work can be removed, by experimenting with fatigued persons. The reviewer has been studying the comparative effects of anoxemia and of continuous mental work on performance. They were found to be similar; i.e., an hour's mental work produces the same decrement in performance level that results from breathing air containing only 10.5 per cent oxygen. Also, output decrement is very rapid under anoxemic conditions. This suggested that a fatigued worker might benefit from breathing pure oxygen. A study was therefore performed in which subjects, fatigued from a half hour of mental work in normal atmosphere were given pure oxygen for fifteen minutes while continuing to work. The results indicate a recuperative effect of the oxygen but are too incomplete to justify any conclusion as yet.

Still another attack on the problem from a different angle is that of Shock (38). He altered the blood chemistry of his subjects within wide limits of alkalinity and acidity by administering the appropriate doses of soda bicarbonate or ammonium chloride to determine whether either their level of output or feelings of fitness for work would be affected. He found feeling-tone greatly affected, but output not reliably altered.

C. *Changes in objective output.* As compared with the rapid decrement in performance shown in tests of continuous muscular work of isolated muscle groups such as are involved in the Mosso ergograph, for

example, the decrement in output in mental work is relatively small over long periods of time. The characteristic form of the work curve shows a rapid adjustment during the early stages from the initial high level to a lower level which the worker can maintain almost indefinitely without a complete breakdown. The classic example is Arai's (1) twelve-hour work curve for continuous mental multiplication of four-place by four-place numbers, which showed a total decrement to about one-half her initial level. However, later studies have shown the amount of decrement in mental work to vary with the nature and difficulty of the task. Where tasks were used of varying degrees of homogeneity, it was found that highly homogeneous tasks produce much more rapid decrements than do varied or heterogeneous tasks (32). The law of homogeneity is that: the more homogeneous the task, the greater the decrement. This effect is possibly accounted for in terms of the neurophysiological principle of cumulative refractory phase, or the tendency for the post-stimulation refractoriness of an arc to be prolonged after repeated rapid stimulations. In homogeneous work, a limited system of neural arcs is repeatedly stimulated. This is probably the objective counterpart of what is subjectively known as "monotony." Closely related to the principle of homogeneity is that of *continuity*. The more continuous the task, i.e., the more rapidly the successive stimulations follow one another, the greater the decrement. If the task is sufficiently discontinuous, no decrement will occur. A third principle, however, that of interference or competition between the elements in the task, works directly opposite to the homogeneity law. If the response tendencies excited by the task are antagonistic to one another, then the decrement is proportional to the number of such competing elements. This reverses the homogeneity law. For example, in the color naming task, where the subject has to continuously discriminate color stimuli, the decrement in the work curve has been found to be proportional to the number of different colors used in the series (6). That is, by increasing the number of possible wrong responses, we make it more difficult for the correct response to occur, because the number of inhibitory tendencies must be increased with a resultant greater drain on the neural resources. A related phenomenon is the increased decrement which occurs in tasks which require a rapid "shift of set" on the worker's part. For example, if a subject must rapidly alternate every few seconds between addition and subtraction of numbers, a greater decrement will result than from performing either one exclusively.

Mental work involving the operation of firmly established habit sys-

tems suffers less fatigue decrement than work involving unpracticed habits. This is true of the organic state as well as the output (34) (33). This principle is probably closely related to the interference principle already mentioned. When new habit systems are set in operation, there occurs a wide spread of excitation into many irrelevant channels. As the habit is strengthened by practice, these irrelevant reactions drop out. The performance becomes more economical, and hence, less fatiguing to the whole sensory-neuro-muscular mechanism. These, then, are some of the main characteristics of the mental work task which determine the amount of fatigue decrement, i.e., homogeneity, continuity, competition, and stage of practice. Robinson has formulated seven principles of the work decrement, including these. No doubt other principles will develop from further studies. For example, does *complexity* in itself make mental tasks fatiguing? Does the fact that multiplication of four-place numbers is more complex than multiplication of two-place numbers make the former more fatiguing? Subjectively, the former task is harder, yet objectively it probably produces no more decrement.

Individual differences. All students of the mental work curve are agreed in finding wide differences between individual subjects in the general form of their work curves. But the attempt to assign particular types of curve to particular persons as characteristic of them has been unsuccessful because of the variations from task to task. Any attempt to write a generalized equation for the work curve is handicapped by these facts.

The blocking phenomenon. In addition to the decrement in speed and quality of output resulting from continuous mental work, there is a very significant change which takes place in the character of the performance which has an important bearing on the true nature of mental fatigue. The reviewer, in an attempt to discover why mental work yields such small decrements as compared with muscular work, made an intensive study of each separate response in a continuous work record of color naming, adding digit pairs, naming opposites, code substitution, and other repetitive tasks (5). The exact time of each response was kymographically recorded. It was found that the performance is not continuous (even though the worker tries to make it so) but is interspersed with short pauses occurring about three times a minute, on the average. These pauses or blocks are as long as two or more average response times and increase in length and frequency throughout the work period. They are apparently due to some cumulative refractory phase in the

response mechanisms which furnishes frequent rests and enables the worker to continue at his original rate. In fact, if the normal response times (exclusive of the "block" pauses) are computed, it is found that there is no decrement in reaction time after an hour's work. The increase which occurs in the length and frequency of the blocks gives the worker enough rests to compensate for the fatigue which would otherwise develop.

When the homogeneity of the task is systematically varied, the blocks vary concomitantly in frequency and length (6). Practice in the task reduces the blocking tendency just as it has been found to lessen the fatigue effect. If the rate of speed at which the subject works is automatically controlled by the experimenter, and tests are made at increasing speeds, there is found to occur a positively accelerated increase in the frequency and length of the blocks (8). A study of the relation between blocking and errors shows that there is a consistent tendency for errors to occur in connection with blocks, either directly before or directly after. The assumption is that the same common factor is responsible for both, i.e., a period of lowered reactive capacity which recurs at frequent intervals, and serves a recuperative function.

Transfer of fatigue. According to present day theories and findings concerning the "mass" functioning of the cortex, mental work must be assumed to involve not only the limited sensory-neuro-muscular systems immediately concerned in the specific intellectual operations, but also the general facilitative action of the entire cortex. Besides, there are many specific operations which occur in more than one kind of mental work. Thus the same digits occur in addition, subtraction, division, multiplication, etc. Hence, we can assume that the fatigue which develops as a result of continuous work on one mental task should carry over to any other subsequent mental performance, and that the degree of such transfer should be in part proportional to the degree to which the two tasks involve identical or similar operations. But, in part, it should be independent of this, because of the common use of the general facilitative function of the cortex.

Studies of transfer of decrement between mental tasks show positive amounts which vary roughly with the similarity of the tasks. Arai (1) found that nine hours of "mental" multiplication caused a 21.4 per cent increase in time taken to memorize words, and her subjects suffered a 13.5 per cent decrease in ability to memorize nonsense syllables after two hours of mental multiplication; but addition and free association

were unaffected. Chapman (9) compared the amount accomplished by subjects under three conditions—1, continuous addition or continuous cancellation; 2, alternating addition and cancellation every half-minute; 3, alternating rest with either one. The greatest decrement occurred in the continuous condition, the least with the rest, and the alternating task condition gave results about half way between. This was corroborated by Vickery (41). One study employed tasks in which the number of identical elements could be systematically varied. Transfer of fatigue between such tasks was found to be quantitatively proportional to the number of identical elements (7).

There is probably also some transfer of fatigue from muscular work to mental work and vice versa, but the results are very contradictory even on the same person (12). The effect may be facilitative or inhibitive depending on the amount and kind of work performed.

Rest and recovery from fatigue. All three aspects of fatigue, i.e., feeling-tone decrement, organic change, and output decrement, are alike in that they disappear with rest. But the rate of recovery apparently differs. Among the problems raised are these: What are the relative rates of recovery for feeling-tone, organic condition and output? Are they interdependent? How does the rate of recovery compare with that for muscular work? How does it vary with the length of the work period and the difficulty of the work? How should rest periods be distributed for maximum effect? The answers to these questions are very incomplete. Poffenberger (30) found that a ten minute rest period, following five and a half hours of mental work, produced an absolute improvement in feeling-tone which was fairly constant regardless of the type of task or of the amount of previous decrement which had developed. He found no clear relationships between the change in feeling-tone level and the change in output level resulting from rest. As to recovery in organic condition Schubert (36) found, for muscular work lasting up to twenty minutes, that the excess oxygen consumed during the recovery period was a function of the length of the previous work period, and that the rate during the first three minutes of rest exceeded that during work. Thereafter, it gradually fell to the resting level. As to mental work, Rounds, Schubert and Poffenberger (34) made tests of the expired air twice during the recovery period following twenty minutes' work on addition. They found that the metabolic rate had seldom returned to the normal level during several minutes of rest and was frequently higher in the second test than in the first. This

seems to indicate a slower rate of recovery from the organic effects of mental work than from an equal period of muscular work. Is this also true of the output level? Manzer (27) reports that for muscular work of the ergographic type carried on to exhaustion, the average recovery after five minutes was 82 per cent, after ten minutes, 90 per cent, and after twenty minutes, 95 per cent. This gives a negatively accelerated curve, with more than three-fourths of the recovery completed during the first five minutes of rest. No adequate data exist for mental work, but there is some evidence that the rate of recovery is also negatively accelerated. A study should be made in which recovery in feeling-tone, organic state and output level are tested at parallel intervals following a work period.

As regards distribution of rest periods, members of the Kraepelin school, notably Graf (20), have contributed much information. Graf found that, for certain repetitive mental tasks, the optimum rest period was two minutes for each hour of work or five minutes for each two hours, which is considerably less than the corresponding figures for muscular work. The attitude of the worker toward the task and toward the rest is highly important.

GENERAL CONCLUSIONS

On the basis of the evidence available at present we can draw the following general conclusions.

Continuous mental work does produce fatigue effects which are proportional to the length, rate and difficulty of the task, provided difficulty is understood to mean homogeneity and interference rather than mere complexity.

These fatigue effects are manifested in three directions; viz. feeling-tone decrement, output decrement and organic change. There is very little inter-correlation between the amount of change in these different directions.

The organic change is small compared to that from equivalent periods of gross muscular work, and a large part of the organic effect must be attributed to the sensory-motor rather than the neural element of the mental work.

Mental performance is not perfectly continuous but is interspersed with short pauses or blocks which may have a recuperative function because they increase in length and frequency in proportion to the length and difficulty of the task.

The fatigue effects, developed in one mental task, transfer to other mental tasks in proportion to their similarity to it. There is not much transfer of fatigue effects between mental and muscular work.

Recovery from the fatigue effects of mental work progresses at a negatively accelerated rate, occurring most rapidly during the first few minutes. In this, it resembles muscular fatigue.

This review has been confined to the normal manifestations of mental effort and has avoided consideration of results of extreme exertion, such as are popularly supposed to cause serious mental derangements. However, the consensus of psychiatric opinion is against the assumption that sheer intellectual effort, apart from emotional strain, can cause either neuroses or psychoses.

REFERENCES

(1) Arai, T. Mental fatigue. N. Y. Teachers' College Columbia Univ., 1912, 54–115.

(2) Barker, K. H. and M. G. Eye. The reliability of the pH of human mixed saliva as an indicator of physiological changes accompanying behavior. Am. J. Psychol. **47**: 222, 1935.

(3) Benedict, F. G. and C. G. Benedict. Mental effort in relation to gaseous exchange, heart rate, and the mechanics of respiration. Carnegie Institute of Washington, Pub. 446, 1933.

(4) Bills, A. G. The influence of muscular tension on the efficiency of mental work. Am. J. Psychol. **38**: 227, 1927.

(5) Bills, A. G. Blocking: A new principle of mental fatigue. Am. J. Psychol. **43**: 230, 1931.

(6) Bills, A. G. Some causal factors in mental blocking. J. Exper. Psychol. **18**: 172, 1935.

(7) Bills, A. G. and W. McTeer. Transfer of fatigue and identical elements. J. Exper. Psychol. **15**: 23, 1932.

(8) Bills, A. G. and M. Shapin. Mental fatigue under automatically controlled rates of work. J. Gen. Psychol., 1936.

(9) Chapman, J. C. Effect of rapid changes of work on performance. J. Exper. Psychol. **2**: 165, 1917.

(10) Clites, M. T. Certain somatic activities in relation to successful and unsuccessful problem solving. J. Exper. Psychol. **18**: 708, 1935.

(11) Day, M. E. Influence of mental activities on vascular processes. J. Compar. Psychol. **3**: 333, 1923.

(12) Dockeray, F. The effects of physical fatigue on mental efficiency. Kansas Univ. Science Bull. **9**: 197, 1915.

(13) Dodge, R. Mental work: A study in psychodynamics. Psychol. Rev. **20**: 1, 1913.

(14) Forbes, A. and L. H. Rice. Fatigue in peripheral nerve. Am. J. Physiol. **90**: 119, 1929.

(15) FREEMAN, G. L. Mental activity and the muscular processes. Psychol. Rev. **38**: 428, 1931.
(16) GERARD, R. W. Studies on nerve metabolism. Am. J. Physiol. **62**: 381, 1927. Also Physiol. Reviews **12**: 469, 1932.
(17) GILLESPIE, R. D. The relative influence of mental and muscular work on the pulse rate and blood pressure. J. Physiol. **58**: 425, 1924.
(18) GOLDSTEIN, H. A biochemical study of the metabolism of mental work. Arch. Psychol., **24**: 1, 1934.
(19) GOLLA, F. L. Respiratory mechanisms in relation to thought. Brain **52**: 491, 1929.
(20) GRAF, O. Rest pauses. Kraepelin Psychol. Arbeiten, **7**: 548; **8**: 265; **9**: 228, 563, 1925.
(21) GROLLMAN, A. Effect of psychic disturbances on cardiac output. Am. J. Physiol. **88**: 584, 1929.
(22) HARMON, F. The effect of noise upon certain psychological and physiological processes. Arch. Psychol. **23**: 147, 1933.
(23) HEFTER AND JUDELOWITSCH. Biochemical changes in the organism during fatigue. Biochem. Ztschr. **193**: 62, 1928.
(24) JACOBSON, E. Electrical measurements of neuro-muscular states during mental activities. Am. J. Physiol. **91**: 567, 1930; **94**: 22; **95**: 695, 703; **97**: 200, 1931.
(25) KNIPPING, H. W. The respiratory exchange, blood reaction and level of phosphoric acid in the blood during mental work. J. Biol. **77**: 165, 1923.
(26) LENNOX, W. G. The cerebral circulation XV. The effect of mental work. Arch. Neurol. and Psychiat. **26**: 725, 1931.
(27) MANZER, C. W. An experimental investigation of rest pauses. Arch. Psychol., **16**: 1, 1927.
(28) MUSCIO, B. Is a fatigue test possible? Brit. J. Psychol. **12**: 31, 1921.
(29) MYERSON, H. AND R. D. HALLORAN. Studies of the biochemistry of the brain by internal jugular puncture. Am. J. Psychiatry **10**: 389, 1930.
(30) POFFENBERGER, A. T. Effect of continuous work on output and feelings. J. Applied Psychol. **2**: 459, 1928.
(31) ROBINSON, E. S. Work of the integrated organism. Chap. 12 in C. Murchison's Handbook of General Experimental Psychology, Worcester, Mass., 1934.
(32) ROBINSON, E. S. AND A. G. BILLS. Two factors in the work decrement. J. Exper. Psychol. **9**: 415, 1926.
(33) ROBINSON, E. S. AND F. R. ROBINSON. Practice and the work decrement. Am. J. Psychol. **44**: 547, 1932.
(34) ROUNDS, G. H., H. J. P. SCHUBERT AND A. T. POFFENBERGER. Effects of practice upon the metabolic cost of mental work. J. Gen. Psychol. **7**: 65, 1932.
(35) ROUNDS, G. H. AND A. T. POFFENBERGER. The measurement of implicit speech reactions. Am. J. Psychol. **43**: 606, 1931.
(36) SCHUBERT, H. J. P. Energy cost measurements on the curve of work. Arch. Psychol. **21**: no. 139, 1932.

(37) SEARS, R. Psychogalvanic responses in arithmetical work. Arch. Psychol. **24**: 1, 1933.

(38) SHOCK, N. Mental performance and the acid base balance of the blood in normal individuals. Proceedings of Indiana Academy of Sci. for 1930, Vol. **40**.

(39) TARANOWITSCH, L. E. Influence of mental fatigue on the excretion of organic phosphorus in the urine. Biochem. Ztschr. **194**: 461, 1928.

(40) THORNDIKE, E. L. The curve of work. Psychol. Rev. **19**: 165, 1912.

(41) VICKERY, K. The effects of work on the work decrement. J. Exper. Psychol. **14**: 218, 1931.

REVERSIBLE INACTIVATIONS OF CERTAIN HYDROLYTIC ENZYMES

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Contemporary investigation in enzymic catalysis is developing new approaches to the solution of difficult problems. These promise to be of additional service in the attack upon certain related biological fields. An outline of the most recent progress would include 1, the isolation of crystalline proteins so closely identified with enzyme activities that they have been designated crystalline enzymes (111) (85); 2, the identification of certain other more or less well-defined organic substances as participants in bio-catalysis;[1] 3, a more penetrating analysis of the specificities *a*, of proteolytic enzymes (5) (made possible by significant advances in the separation of enzymes (130) and in the synthesis of peptides), and *b*, of the cellular dehydrases; and 4, the extension of our knowledge of the several physical and chemical factors controlling catalysis by enzymes. Such categories are interrelated. A portion of the last named field has been assigned as the subject of this review.

There will be emphasized recent studies which have permitted a correlation of the control of activity of several hydrolytic enzymes with *reversible* chemical processes such as oxidation-reduction, the action of mercaptide-forming metal compounds, or metal ion coördination. A comprehensive treatment of the numerous isolated studies of enzyme activations and inactivations will not be attempted. Reference will be made occasionally to the bearing of methods and of interpretations upon problems in adjacent fields.

Activation of enzymes. In a series of papers, now classic, Sørensen (110) demonstrated the necessity for and set forth the means of securing in enzyme studies an adequate control of what is now designated the hydrogen ion activity (14). The relation of this variable to enzyme

[1] For example, Warburg's "yellow respiratory enzyme" (protein-phosphoflavin) (126) (116) (62); the coenzyme of red blood cells (127) and cozymase (27); cytochrome-*C* (135). Note (66a).

action was studied intensively by Michaelis and his co-workers (77) who employed in their analysis the concept of the ampholyte character of enzymes and of enzyme-substrate intermediates. Activity-pH curves (for specified temperatures) are now evaluated as a first step in the description of an enzyme activity and illustrate the most obvious method of attaining a reversible activation (compare the case of pepsin (85)). Irreversible inactivations of enzymes by H^+ and OH^- ions complicate the picture, and are treated generally as destructive chemical actions upon the fragile enzyme molecules (40).

In addition to those changes in ionic species which are brought about by pH-control (e.g., by the use of buffers) the activity of enzymes in general may be altered through various "salt effects." The most *general* kind of salt effect would result from a change in the thermodynamic environment resulting from the presence (14) in reaction mixtures of added ions or molecules (buffer or other salts, amino acids, proteins, etc.). The magnitude of general salt effects may, perhaps, be gauged by studies of the variation of enzyme activity with the ionic strength (65) of the medium. There is needed systematic work upon this point. The experiences of investigators (12) in other fields of catalysis should, however, be taken as a warning concerning the probable complexity of the matter (see 34).

Consideration of some of the larger, more *specific* effects is included in this review. In the literature (40) are to be found descriptions of a bewildering array of enzyme activations and inactivations by various reagents. The activating effects of sundry reagents have been interpreted in terms of coenzyme actions, salt effects, modification of electronic characteristics of the enzymes, or changes in oxidation-reduction state of their specific activators, removal of inhibiting metal ions, etc. Correlation and rationalization are emerging nevertheless from the rich background of experimental material. This may be illustrated by a severely restricted historical survey. Here and elsewhere reference to the literature remains regrettably incomplete. Fortunately, there are now available some excellent descriptive reviews. (For example (84).)

Catheptic proteinases; papain. Observations akin to the depression of catalase activity by HCN (102) are of long standing. Of more recent origin are consistent studies of the specific effects of certain reagents upon so-called hydrolytic enzymes like urease, arginase, phosphatases or papain. Vines (120) attempting the use of cyanide as a disinfectant in studies of papain, the proteolytic enzyme of the pawpaw (*carica papaya*), encountered in hydrolyses of gelatin an unanticipated, markedly *favor-*

able action of this reagent. That this effect involved an "actual acceleration of proteolysis" was brought out by the experiments of Mendel and Blood (74), who also discovered similar behavior in the case of H_2S, but not of a third weak acid, HBO_2. Later, Willstätter, Grassmann, and Ambros (131) with improved experimental methods greatly extended the scope of investigation. The results of none of these studies seemed to justify an interpretation of the activations in chemical terms. However, the observations indicated generally that the action of HCN or of H_2S resulted primarily in an enhancement of the potency of the catalyst, *corresponding virtually to an increase in the enzyme's concentration.* According to Frankel (30) no HCN is destroyed in the course of digestion and this precludes its action as a reducing agent. However, the analytical methods available to him were probably incompetent to decide this question.

The existence in certain plant tissues of material ("phytokinase") similar to HCN in its action upon papain and bromelin (papain-like enzyme of pineapple) was first suggested by the work of Willstätter and his students, particularly Ambros and Harteneck (2). Waldschmidt-Leitz and co-workers, were able to show (122) that proteolytic enzymes (cathepsins) of certain animal cells (e.g., of liver) not only resemble the papain-type plant proteinases as regards their activation by HCN or H_2S, but likewise seem to be associated in the tissue with activators ("zoökinase"). The tripeptide, reduced glutathione (50), was identified as such a naturally occurring activator for yeast protease by Grassmann et al. (38) and for liver cathepsin by Waldschmidt-Leitz, Purr and Balls (124) after it had been shown (37) *that cysteine and HS-glutathione are as characteristic in their enhancement of the proteolytic potency of papains and of cathepsins as HCN.* Such observations naturally brought forth speculation concerning the rôle of activators in enzymic catalysis as well as of the *control* of enzyme action *in vivo* (36).

Suppression of enzyme activity by metal ions and by other reagents. The "poisoning" action of certain salts (e.g., $AgNO_3$, $HgCl_2$) upon enzymes is too well known to require emphasis. It has been shown that the inhibiting effect of silver ions upon saccharase (25) or crystalline urease (111) is fully reversed by the action of H_2S. The silver-saccharase effect was considered by Myrbäck (82) to involve a dissociable silver salt of the amphoteric enzyme. Krebs (60) examined the marked sensitiveness of papain to the action of certain heavy metal ions. He sought to interpret the activating qualities of HCN and of H_2S, etc., in terms exclusively of the removal of inhibiting metal ions. In contrast, it is

well established that heavy metal ions are not invariably inhibitors. (Compare (79).) Ferrous ion *activates* arginase. Furthermore, catalysis by hydrolytic enzymes is not always favored by HCN. The activity of certain peptidases, plant proteases, or arginase is suppressed under well-defined conditions by HCN, H_2S or cysteine.

The conflict in current views of the nature of enzymes is reflected in a certain confusion in the treatment of activations, inactivations, coenzyme and other effects. However, it is now recognized that catalase, peroxidases, cytochrome, and certain oxidases are probably all hemochromogen types (cf. (56)). Their structures would be comparable to hemoglobin in consisting essentially of a more or less specific protein combined with a component (a heme) in which a metal atom is coordinated in part with a porphyrin nucleus. This provides the basis for a sound treatment of the suppression of activity of these catalysts by isocyanides, HCN, H_2S, CO, and many other reagents. It also implies a mode of control of the pace of certain oxidative processes through the interplay of metal-coördinated complexes. In another field, the crystallization of urease by Sumner and of pepsin, trypsin, chymo-trypsin and their precursors by Northrop has made available materials of unique value in chemical studies, including activation. These protein-enzymes are astonishingly homogeneous and are, of course, free from "accompanying" material which is always present in crude enzymes and may modify their behavior. Intensive study of such products is particularly to be desired for an appreciation of the kinds of processes available to the organism for the control of enzyme activity.[2]

Urease. An investigation of the properties of crystallized urease in relation to the control of action of this enzyme did, in fact, lead to the resolution of difficulties and to a clear-cut definition in chemical terms of several aspects of enzyme activation. The essential problem is revealed in an account of some of the older studies. Until recently studies of urease were made with more or less crude preparations derived from de-fatted soya or jack bean meal. The preparation of Van Slyke

[2] Trypsinogen is converted to trypsin autocatalytically (85); the process may involve a slight proteolysis of the precursor. The autocatalytic activation of trypsinogen (induced by an intial trace of trypsin) recalls "the propagation of bacteria or of filterable viruses." The conversion of chymotrypsinogen to chymotrypsin, induced by trypsin, proceeds monomolecularly. Vexing questions concerned with trypsin inhibitors and the rôle of enterokinase (63) apparently are yielding to the newer methods. Reversible inactivations of pepsin and of trypsin which are clearly dependent upon the protein nature of these enzymes have been described by Northrop.

and Cullen (see 88) admirable as reagent catalyst in estimations of urea, contains impurities which mask certain properties of the enzyme itself.

Urease, even in the crude mixtures, is more or less sensitive to the action of a number of salts; for example, those of silver, mercury and copper. The literature is summarized in general references (86) and in the papers by Jacoby (52), Kitagawa (57), Ruchelmann (97) and others. Inactivations by metal ions are often reversible with H_2S. Iodine is injurious (in accordance with usual experience in enzyme work) and cysteine, thioglycollic acid ($HS\text{-}CH_2\text{-}COOH$), pyrophosphate and KCN are reported to enhance activity. The response toward various reagents of the highly specific enzyme, urease, thus bears a formal resemblance to that outlined for papain and similar "intracellular" proteinases. In numerous studies there was not recognized the possible intervention of an ever present reagent, the oxygen of the air. For this and other reasons, the interpretation of observations especially with metal ions has been rendered difficult. Because of varying conditions under which observations were made the results of different authors often defy evaluation.

Crystalline urease. The inhibition of activity by metal ions is accentuated in crystalline urease. This product must be brought into solution with water, redistilled in glass, instead of ordinary distilled water because of traces of heavy metal impurity in the latter. Sumner and Myrbäck (see 111) found that the purified enzyme is protected against the action of heavy metal ions by H_2S, proteins, amino acids, phosphate buffer, and, in general, substances that combine with or adsorb such cations. A similar protective rôle is displayed, also, by certain materials derived from the plant tissues which accompany urease in crude preparations.

It was noted by Perlzweig (87) that a weakening of the nitroprusside test for thiol (-SH) groups accompanies partial inactivation of crystalline urease during aeration. Perlzweig suggested that association of urease with a sulfhydryl activator (possibly glutathione) would account for the reactivation by cyanide, hydrogen sulfide and other reagents of preparations "poisoned" by the addition of ions of heavy metals. On this basis the "poisoning" would consist of the metal-ion catalysis of oxidation of the activator, regeneration of which might result from the reducing action of the cyanides or sulfides. However the work of Sumner and Poland (112) shows very definitely that although sulfhydryl compounds such as glutathione may be present in jack bean meal, crystallized urease does not contain glutathione or other extractives

which respond to nitroprusside. Urease which has been repeatedly recrystallized and dialyzed possesses characteristic activity in the absence of supplementary thiol compounds. On the other hand, the protein-urease molecule *itself* contains a group, probably sulfhydryl, which gives a nitroprusside test. Moreover, constancy in degree of response to the test for thiol groups accompanies constancy of ureolytic activity when crystalline urease is recrystallized. The *highly purified* preparation, essentially free of heavy metal contaminants, is little sensitive to the action of air.

REVERSIBLE INACTIVATION OF CRYSTALLINE UREASE. Hellerman, Perkins and Clark (43) proposed that some of the phenomena associated with the effects of reagents upon papain, cathepsins, urease, etc., might be ascribed, not to changes in a supplementary activator, but more directly to the oxidation of and regeneration of the substituent sulfhydryl groups of the enzyme itself. The enzyme would then be catalytically active in its reduced form, e.g., En-S-H, and reversibly inactivated by controlled oxidation to an inactive form, En-S-S-En. This working hypothesis was tested first with crystalline urease.

On this basis, reversible inactivations might be accomplished not alone by oxidations but, in general, by reversible chemical actions upon thiol groups of the enzyme molecules; for example, by the use of salts or derivatives of metals that readily transform mercaptans (R-S-H) to mercaptides (R-S-Me). Examples of such reagents are silver-ion, mercuric salts, cuprous oxide (50) (89) and aryl- or alkylmercuric salts (98), or hydroxides (e.g., C_6H_5HgOH). The reaction involving cuprous oxide is illustrated by the equation

$$2\ \text{R-S-H} + \text{Cu}_2\text{O} \rightleftarrows 2\ \text{R-S-Cu} + \text{H}_2\text{O}$$

Representing as Me^+, any effective univalent cation, one would write, more generally,

$$\text{R-S-H} + \text{Me}^+ \rightleftarrows \text{R-S-Me} + \text{H}^+$$

Similarly, there would result from the action of a substituted mercuric salt or hydroxide (R'HgX, where X = Cl or OH), a product of the type, R-S-Hg-R'. It is readily seen that pH control is a factor in such actions; also that partial or complete reversals would be accomplished by reagents that in one way or another remove or bind the effective metal ions (e.g., H_2S, HCN, mercaptans, amino acids, proteins, etc.).

This at once offers a partial explanation for the "protective" effect

of many substances, including the supposed co-enzyme action of "auxo" substances like amino acids or HCN (see 111).

Potent preparations of crystalline urease in appropriate solutions were treated (43) with a series of organo-mercurials (129) of the type, RHgX, including phenylmercuric chloride or hydroxide, benzylmercuric chloride or chloromethylmercuric chloride ($ClCH_2HgCl$ (42)). In all cases the ureolytic activity was rapidly wiped out. These effects were shown to be reversible. In each case studied, there was observed extensive or complete recovery of enzyme activity after the addition to the inactivated urease solutions of such reagents as H_2S, HCN or certain mercaptans. In later work (46) with papain it was found that similar inactivations by phenylmercuric hydroxide (or Ag^+-ion) were reversed, in addition to the reagents stated, by bromide-ion, which alone did not significantly alter the activity. The bromide-ion could conceivably act by formation of slightly soluble silver bromide or phenylmercuric bromide or "stable" complex ions (e.g., $AgBr_2^-$). Inactivation by cuprous oxide, and reactivations from the action of this reagent were also found to be very characteristic. The possibility of inactivations by adsorption of the enzyme upon the surfaces of slightly soluble metal compounds used was apparently excluded in these experiments. It is important to note that symmetrically substituted mercury compounds such as bis-iodomethyl mercury (ICH_2-Hg-CH_2I) or dibenzyl mercury, which cannot directly convert mercaptans to substituted mercaptides, were found incompetent to inactivate urease.

Consonant with these observations were the characteristics of inactivation by oxidizing agents and reactivation by reducing agents. Representative thiol compounds are known to display marked differences in their sensitiveness toward attack by oxygen. These are essentially differences in reaction rates. In general also, the presence of certain heavy metal-ion impurities markedly affects the rate of oxidation (for references, see 32). Highly purified cysteine is but slowly attacked by oxygen, while this substance and other thiol compounds are rapidly oxidized in the presence of ions of iron and especially copper. Hellerman, Perkins and Clark found that several hours' vigorous aeration of solutions of crystalline urease (or treatment of the enzyme with hydrogen peroxide) reduced the activity only slightly (about 10 per cent). The slight activity loss was readily restored with HCN or H_2S. On the other hand, in the presence of small, specified amounts of cupric-ion and under controlled conditions, the ureolytic activity was abolished by aeration for $\frac{1}{2}$ hour. Restoration could be accomplished in large meas-

ure by the addition to solutions of inactivated urease of such reagents as thioglycollate-ion, cysteine (not cystine), neutralized, reduced glutathione, H_2S or HCN. Aeration in the presence of Cu^{++}-ion for longer periods resulted in the irreversible inactivation of urease. The effect of ions of iron was much less pronounced than of copper. Although the effect of Cu^{++} was plausibly attributed to catalysis of the enzyme's oxidation, the possibility of a more specific action of the metal ion (e.g., formation of a cupric or, by reduction, a cuprous complex) was not excluded. However, the ions, Co^{++}, Ni^{++} and Mn^{++} do not greatly affect the ureolytic activity of crude urease (100), and this has also been found (46) to be true for crystalline urease.

Sulfhydryl compounds may also be oxidized smoothly to their dithio derivatives by iodine. Iodometric estimation of such compounds is a standard procedure. The inactivation of urease by iodine under conditions so controlled that extensive reversals might be obtained by suitable subsequent reduction would be peculiarly suggestive. Such changes were in fact observed with solutions of crystalline urease, the conditions being so arranged that the observations were satisfactorily reproducible.

Irreversible inactivations with iodine were, of course, readily obtained. While suitably controlled oxidation of a mercaptan, R-S-H, by iodine, oxygen, or ferricyanide may be expected to yield the corresponding dithio derivative, R-S-S-R, from which, in turn, the mercaptan is recovered by appropriate reduction, more drastic changes are readily effected. For example, the oxidation of sulfur atoms to a stage beyond the dithio configuration would, in the present sense, be essentially irreversible. In the case of urease iodine might also substitute upon various groupings of the protein molecule. Such actions would be reversible to a certain extent, but this aspect need not be elaborated here.

REVERSIBLE INACTIVATION OF PAPAIN. EFFECTS OF OXIDATION-REDUCTION AND OF METAL COMPOUNDS. The plant proteinases, designated papains, are apparently enzyme mixtures. There is present in papain of the pawpaw, for example, the familiar cyanide-activatable catalyst which accelerates splitting of gelatin, fibrin, etc. (and, when activated, peptones and protamines (?) (2)), and, in addition, peptidases which act upon lower split-products. These peptidases seem to be *inhibited* by HCN (2); this will be discussed later. Here we shall use "papain" in its customary sense, viz., the cyanide-activatable proteinase. It is stated (2) (118) that the degree of activity of papain or bromelin in the fruit of the pawpaw or pineapple, respectively, varies with

the stage of maturity of the fruit, bromelin in ripe pineapple, for instance, being fully active. Commercial papain (36), freshly prepared, has strong proteolytic activity which may, however, be doubled or even tripled by the action of certain "activators." Such material, standing at room temperature, gradually becomes less active; more or less concomitantly it loses the property of becoming activated. This is evidently irreversible inactivation, suggesting a slow spontaneous denaturation, and possibly attack by atmospheric oxygen.

In these observations together with those outlined earlier there is disclosed a certain parallelism in the effects of various reagents upon the activity of papain and of urease. Only shortly after the communication upon urease by Hellerman, Perkins and Clark, there appeared a paper by Bersin and Logemann (9), who found that alcohol-precipitated (131) papain was activated by several sulfhydryl compounds and inactivated by oxidizing agents such as hydrogen peroxide, selenite-ion ($SeO_3^=$), benzoquinone, or iodine. From solutions of commercial papain, treated with hydrogen peroxide at 40°, inactivated enzyme was recovered by alcohol precipitation; such material was readily activated in the presence of glutathione, sulfites, succinate with muscle dehydrase, etc. Bersin and Logemann adopted the working hypothesis that papain, active in a reduced state, may be reversibly oxidized through some oxidizable grouping of the enzyme molecule. Maschmann and Helmert (70) had also made observations which pointed in this direction. They suggested, further, that the activation of cathepsins and papain by HCN might be related to the reduction to thiol of dithio linkages in the protein-enzyme molecules. Bersin accepted this view (7) and reinterpreted his findings in terms of thiol chemistry.

Meanwhile Hellerman and Perkins (44) had been studying the effects of oxidation-reduction and, in addition, of metallic compounds upon papain, with emphasis upon those *reversible processes* which had proved pertinent to the investigation of reversible inactivations of crystalline urease (43). Caution in the interpretation of the results on a sulfhydryl basis seemed advisable because papain, unlike urease, had not been crystallized. Furthermore, preparations which had been partially freed from impurities, for example, by being precipitated from an aqueous solution with ethanol, collected, exhaustively washed with ethanol and dried, were active proteolytically and satisfactorily stable, but responded very weakly to the nitroprusside test for thiol groups. Such groups, however, may have been more or less "masked" (80) with respect to the action of certain reagents. Fortunately, papain under

suitable conditions was found to yield exceptionally definite responses to the action of numerous reagents. The characteristics of inactivation by oxidation, and of reactivation by a number of reducing agents again strongly suggested that *one mode of control* of the proteolytic activity of papain resides in the reversible oxidation of the enzyme, as if the latter, when active, were "a reduced molecule, En-SH, capable of being reversibly inactivated by controlled oxidation to an inactive dithio form, En-S-S-En" (44). As applied to enzymes of the papain type (or urease) this was schematically represented, as follows.

$$\underset{\text{(Active)}}{2\,\text{En—SH}} \underset{\text{(reduction)}}{\overset{\text{(oxidation)}}{\rightleftharpoons}} \underset{\text{(Inactive)}}{\text{En—S—S—En}} + 2(\text{H})$$

In addition to its interesting oxidation-reduction chemistry, papain, like urease, was found extremely sensitive to the action of various mercaptide-forming reagents (e.g., $C_6H_5CH_2HgCl$, C_6H_5HgOH, Cu_2O). Substances that promote the hydrolysis or decomposition of mercaptides effected reactivations. A critical evaluation would weigh with the assumption of mercaptide-formation the general reactivity (129) of the inactivating metallic derivatives toward acids and certain nitrogen-containing groupings; however, the assumption of an almost specific reactivity (under the conditions) of such a compound as benzylmercuric chloride for the supposedly substituent thiol groups seems to accord best with all of the evidence in hand. It is of interest, in this connection, that phenylmercuric hydroxide, applied under similar conditions, does not significantly alter the activity of prothrombin or thrombin (48), insulin (101), or arginase (45).

Details of the application of oxidizing and other reagents and the interpretations of individual observations need not be repeated here. It may be emphasized that reproducible and significant results in this field rest upon adequate and often elaborate controls and, in addition, regard for a number of factors, the importance of which is not always apparent at the first approach. For example, the necessity for control of the temperature and the hydrogen-ion concentration in studies of enzymic catalysis is universally recognized; in addition, the effects of these variables upon the mode and rate of reaction of an added reagent (e.g., ferricyanide) with the enzyme or substrate may require consideration. Again, there is required due regard for general and specific salt effects; e.g., the possibility of interaction between a buffer salt and an added reagent. Moreover, the interpretation of results and comparison

with the findings of other workers must take account of the origin and methods of preparation or purification of enzymes and, so far as possible, of extraneous materials that may be present. The reviewer is convinced that a reasonable resolution of seemingly conflicting observations is often possible.

General experience with papain suggests that commercial preparations and those prepared by alcohol precipitation often contain the enzyme partially in its active state (supposedly "En-S-H") and partially as the form (En-S-S-En) from which activity is elicited by suitable reduction.[3] An important factor is seen in the probability of specific differences in the mode of chemical attack of effective reducing agents as well as of variations in relative rates of reaction. Papain, reversibly inactivated, is remarkably stable in the presence of the substrate, gelatin. Of broader interest is that the activating effects of several reagents suggest a stoichiometric relationship in their action upon papain (44).

As a general summary, there is appended a table which lists some of the reagents the use of which under appropriate conditions results in the reversible inactivation of papain and in addition those that effect reactivations of the resulting inactive material *a*, when the inactivations have been caused by oxidizing agents, and *b*, when they have been brought about by "mercaptide-forming" substances related to or derived from metals.

LIMITATIONS AND SIGNIFICANCE OF THE SULFHYDRYL PICTURE. The conception that reversible chemical actions upon thiol groups of certain enzymes may largely account for their reversible inactivations, not only has its uses in the evaluation of published data, but seems, further, to provide a rational basis for an important mode of *control*, by oxidation and reduction, of the kinetics of several hydrolytic and other processes, *in vivo* as well as *in vitro*. This picture, evidently, is not universally applicable. There is no present reason to anticipate its application to pepsin or to pancreatic trypsin. The over-all transformation of trypsinogens to trypsins seems to consist of an irreversible process

[3] This suggestion and the general discussion may require interpretative criticism in the light of Mirsky and Anson's work (80) on reversible denaturation of proteins containing —S—S— and —SH groupings. This does not in the least impair the utility of the "sulphydryl theory" as a working hypothesis, the validity of which rests upon a considerable body of cumulative evidence with several enzymes (differing markedly in their specificities), in particular the homogeneous protein-enzyme, crystalline urease.

involving a special chemistry (85). This may be true, also, for prothrombin-thrombin. Reversible oxido-reduction is thought to be the essence of function of indophenol-oxidase, and not necessarily of its activation. The hydrolytic enzyme, arginase (irrespective of the methods of its control in the cell) is not readily activated *in vitro* by reduction, in the more usual sense. Its reversible activation by another process will be discussed.

Inactivation and reactivation of papain

Oxidants that inactivate reversibly	*Substances that reactivate after inactivations by oxidizing agents**
Oxygen (aeration in presence of Cu^{++} or Fe^{++})	Reduced glutathione
Iodine	Cysteine (not cystine)
Quinone	Thioglycollate, thiosalicylate, etc.
Hydrogen peroxide	Titanous-ion
Ferricyanide-ion	Hydrogen cyanide
	Hydrogen sulphide
	Succinate plus muscle extract (dehydrase) after H_2O_2
	Ascorbic acid plus ferrous-ion
	[Not bromide-ion (see below)]†
Metal derivatives that inactivate reversibly	*Substances that reactivate after inactivation by metal derivatives*
Phenylmercuric hydroxide (and certain of its salts)	Hydrogen cyanide
Benzylmercuric chloride	Hydrogen sulphide
Chloromethylmercuric chloride, etc.	Reduced glutathione, cysteine, certain thiol acids
Cuprous oxide	Bromide-ion† (after silver-ion or C_6H_5HgOH (46))
Silver-ion; mercuric chloride; cupric-ion (?); etc.	[Not Ti^{+++} (after C_6H_5HgOH; see above)]‡
(Not bis-substituted mercury compounds)	

* Mothes states that papain may be activated by catalysed hydrogenation (81).

† See text under "urease." Bromide-ion would not be expected, under the conditions used, to function as a reducing agent, and as such to reactivate after suppression by oxidants.

‡ Titanous-ion, a potent reducer, reactivates (in citrate buffers) after oxidative inactivation; it also activates papain, prepared according to standard procedures, presumably by reduction of the "dithio-papain" present. It would not be expected to restore that portion of the activity that has been wiped out by the action, e.g., of phenylmercuric chloride. In contrast, H_2S, HCN and thiol compounds not only possess the reductive property, but can, in addition, suppress metal-ion effects essentially through salt-formation.

Two aspects of the sulfhydryl picture seem especially significant. The one involves virtually an extension of the factors governing enzymatic catalysis, as this is observed in the laboratory. To the control of activity through regulation of temperature, pH, more or less general salt effects, etc., is now added, in certain well-defined cases, reversible inactivation and activation by oxidation-reduction (for instance, regulation of the enzyme's oxidation-reduction state). In addition we have the specific ion effects that are beginning to be better understood. This extension of factors is implicit in the experimental structure and theoretical statement of the subject.[4]

Less definitive is the second, and more vital significance, namely, the possibility of existence of an oxidative-reductive mode of control of certain hydrolytic (and, perhaps, other) processes in the living cell. (Compare (81) (121) (134).)

Cerebrosidase. Suggestive for *in vivo* significance are the recent demonstrations of applicability to a number of widely differing fields denoting a more far reaching biochemical importance than was at first contemplated. To the cases of urease and certain intracellular proteolytic enzymes of plant and animal origin may now be added cerebrosidase, representing another type of hydrolytic enzyme which possesses properties apparently fitting the thiol-dithio hypothesis. Thannhauser and Reichel find (115) that cerebrosidase, which is in an inactive state when prepared as an enzyme extract, is efficiently activated by H_2S, cysteine, l-ascorbic acid, or reduced glutathione. Reversible inactivation by oxidation was not demonstrated, but this may tentatively be inferred from the clear-cut nature of the results. Of unusual interest is the additional observation made by Thannhauser and Reichel that those sulfhydryl compounds (e.g., cysteine) that *activate* cerebrosidase markedly suppress the activity of polyaminophosphatidase.[5] The suggestion is advanced that there may exist a reciprocal relationship in the nerve cells with respect to the reversible activation and inactivation of cerebrosidase and the phosphatidase (Cerebrosides and "polyaminophosphatides" or sphingomyelins occur together in such tissue.) It might well be imagined that control of the oxidation-reduction state of cerebrosidase, cathepsin, etc., or of

[4] The matter seems already to have assumed technical importance, for example in baking (53) and in papain assays (114).

[5] An interpretation of such suppression of activity, whether reductive *in*activation or, more likely, some addition reaction (e.g., combination of reduced glutathione with the sphingomyelinase), *etc.*, must await further experimentation.

substances like glutathione which in their reduced state are supposed to *inactivate* the phosphatidase when cerebrosidase is concomitantly activated (presumably by reduction) is vested in the control of the level of oxidation-reduction intensity (see 18) of the cell or pertinent structure thereof. It is worthy of note that Thannhauser and Reichel find in their results a hint regarding the pathogenesis of Gaucher's disease, Niemann-Pick's disease and of the amaurotic idiocy.

Relationship in the regulation of papain and papain-peptidase. The reciprocal relationship referred to in the preceding section is remindful of certain observations (2) upon papain and the peptidase (hereafter designated papain-peptidase) which accompanies papain in crude papaya latex. The characteristics of these enzymes have recently been the object of renewed study by Bergmann and his co-workers, who, by virtue of their elegant method of polypeptide synthesis (5) were provided with a large variety of substances upon which to test the specificity of the peptidase. It appears that there are actually present in each preparation of the papain type (e.g., papain, bromelin, animal cathepsin) which has been studied at least two catalytic activities, viz., the cyanide-activatable proteinase (which "splits" gelatin, fibrin, etc.) and peptidase which can act upon such synthetic substrates as carbobenzoxytriglycyl-l-leucyl-glycine or hippuryl amide. The proteinase (called "papain" in this review) and peptidase ("papain-peptidase") are designated by Bergmann et al. as papain peptidase II and papain peptidase I, respectively. It is obvious that the investigations by Bersin and by the reviewer were concerned chiefly with the reversible inactivation of papain (Bergmann's peptidase II) and not with papain-peptidase. The work of Bergmann et al. has revealed interesting and important peculiarities in the specificity of papain-peptidase as compared with carboxy- and amino-polypeptidases previously studied. Their results upon activation do not contradict in any way the observations of previous investigators upon the regulation of papain (proteinase) activity by oxidation-reduction, but they do seem to the reviewer to indicate clearly that certain reagents which can activate papain are also effective inhibitors of papain-peptidase. Chiefly on the basis of observations of the effect of phenylhydrazine upon these enzyme actions, Bergmann suggests that papain and papain peptidase mutually inactivate each other by combining (reversibly) to form an addition compound; further, that the peptidase may contain an aldehyde grouping, through the medium of which HCN, H_2S, sulfhydryl compounds, and particularly phenylhydrazine cause dissociation of the proteinase-

peptidase complex, freeing and hence activating proteinase papain. Unfortunately, however, the action of phenylhydrazine is not necessarily a specific one; thus it binds carbonyl compounds and can function also as a reducing agent or as an oxidizing agent, depending upon the conditions. As a derivative of hydrazine it enters into complex formation with certain ions or other metallic derivatives; such a combination might be reversed by the action of other substances (e.g., benzaldehyde) that react with phenylhydrazine. Hydroxylamine was found to inactivate both papain and papain-peptidase. Pyridine caused inactivation of neither under the conditions. Such considerations illustrate the difficulty of interpretation of much of the activation work. Complications are introduced also by the use of different preparations of papaya latex containing various enzymes and accompanying substances. On the basis of all of the evidence now available a conservative appraisal would reject any *ad hoc* interpretation of the activation of papain-peptidase pending further investigation of the chemical nature of its reversible activation. In the control of activity of proteinase and of peptidase there may well exist a reciprocal relationship, as suggested by Bergmann; the characteristics will probably be decided by further experimentation. The occurrence of several related catalytic activities controlled by differing and reciprocal mechanisms would be of much interest for the coördinations of cellular function. Although cyanide, *inter alia*, might to some extent *activate* either enzyme (in crude mixtures), whether by removal of heavy metal inactivants or by reduction, it is altogether likely that such a reagent would elicit a different response in a purified papain-peptidase (cf. *Arginase*).

Hemolysins; lysozyme. Neill showed (83) that pneumococcal hemolysin which has been inactivated by air or by H_2O_2 may be reactivated by the use of reducing conditions. This phenomenon bears a marked resemblance to the activation picture for urease and papain; Shwachman, Hellerman and Cohen undertook (107) a study of the effects of oxidation and reduction as well as of mercaptide-forming metallic compounds upon this lysin and were able to demonstrate a remarkable parallelism in the regulation of activity of the lysin and of the hydrolytic enzymes named. The lysin is active when reduced, and may be *reversibly* inactivated by operations that, under suitable conditions, are known to convert sulfhydryl compounds to the corresponding dithio derivatives or to mercaptides. Cohen and Shwachman (19) have examined the relationship of these observations with other mechanisms

of inactivation, notably that which involves the use of certain sterols. They find also that the reversible inactivation of hemolysin by the oxidants of a series of dye systems occurs only when the E_0' (at pH 7.6) of the dye system is more positive than -0.07 volt. These data indicate the zones of oxidation-reduction intensity in which the lysin displays activity, and, in addition, may aid in establishing an apparent level of reduction intensity characteristic of the hemolysin. The latter was obtained from pneumococci, type II, but the phenomena are not restricted to this particular hemolysin.

Meyer, Thompson, Palmer and Kharazo (75) tested lysozyme (29) of egg white with similar methods and concluded that the lysozyme also is a sulfhydryl-containing entity which is active only in the reduced state. They indicate the similarity in their results with those of Shwachman, Hellerman and Cohen and suggest a possible rôle of peroxides in certain bacteria as a defense against lytic substances.

Little is known regarding the nature of the linkages attacked by these lytic agents. Striking, however, is the analogy in the effects of oxidation-reduction and metallic derivatives upon these biological catalysts on the one hand and upon hydrolytic enzymes (urease, papain) possessing well-defined specificities, on the other.

Related problems in various other fields. That oxidative-reductive control may be a factor in glycolytic mechanisms was indicated by the work of Quastel and Wheatley (94) and of Lipmann (67). This would hold much of interest for the interpretation of diminished glycolysis in the Pasteur effect (see 49). Whether a "sulfhydryl-enzyme" is involved is a question for further critical inquiry in a very difficult field. Reference may be made here to studies (68) (54) (133) of the rôle of reduced glutathione in the enzymatic dismutation of methylglyoxal (to lactate), the position of which in glycolysis (or general carbohydrate metabolism) remains uncertain. The enzyme-catalyzed process is thought to involve an initial specific addition of this thiol compound to a carbonyl group of the substrate. Such hemi-mercaptal formation is receiving close study (103).

It was shown by Goddard and Michaelis (35) that the correlations of enzymatic hydrolysis and oxidation-reduction may directly concern substrate as well as enzyme. In the presence of proteolytic enzymes the hydrolytically-resistant keratins are readily attacked after there has been effected a suitable preliminary reduction, presumably of dithio groupings substituent in the keratin molecules. Linderstrøm-Lang and Duspiva observed (66) that there exists in the digestive

tract of the clothes-moth larva a suitable level of oxido-reductive intensity for this process. Wool keratin is thus reduced, antecedent to the digestive action (at pH 9.3) of a potent proteinase which itself is "stable" under the rather extreme conditions.

Studies of the effects of reducing agents upon several *endocrine principles* have to the present yielded little of interest for *reversible* inactivation. It was shown by du Vigneaud (119) that the hypoglycemic effect of insulin is abolished by the action of sulfhydryl compounds upon the hormone. The effect is essentially irreversible (compare 31); it has a bearing nevertheless upon the structure of insulin molecules and may become more directly of physiological moment. None of the sulfur of protein-insulin seems to be in the sulfhydryl state. Apparently, those thiol compounds (101) which activate urease and papain, presumably by reduction of dithio linkages in the reversibly inactivated enzyme molecules, are also able to *inactivate* insulin. Conversely, thiolglyoxalines (e.g., ergothioneine) which do not readily activate papain are incapable of inactivating insulin.

Gulland and Randall have studied the action upon the posterior pituitary's oxytocic principle of certain reducing agents, including the reductants of various oxidation-reduction indicator dyes, and believe their results have disclosed a relationship between the activity of the endocrine and its oxidation-reduction state (39). They do not, however, feel justified in suggesting definitely that a disulfide linkage is present in their active (and supposedly oxidized) material. Sealock and du Vigneaud's recent investigation (106) involved a different approach which led them to conclude that pitocin may be reduced by cysteine without substantial impairment of its activity, and, indeed, that substituent sulfhydryl groups or "potential" sulfhydryl groups (i.e., -S-S-) are essential to the hormone's activity. It would appear that the validity of the differing interpretations must rest upon studies with highly specific methods and possibly the availability of the pure hormone.

Critique of methods and deductions. The observations with oxidizing, reducing, and certain other reagents have been treated above in terms of a tentative but highly plausible hypothesis. This assumes that the effects may be attributable largely to reversible chemical actions upon thiol groups which may be considered to be integral substituents of the molecules of the specified enzymes. It is not surprising, therefore, that the correlation of oxidation-reduction with bio-catalytic activity in the cases reviewed has had to be presented in very specific terms with

little reference to the oxidation-reduction potentials of the systems involved. It was emphasized by Hellerman and Perkins (44) that systems indisputably of the sulfhydryl-dithio type, although in a broad sense oxidation-reduction systems, have not yet been shown *experimentally to come into equilibrium rapidly* with such strictly reversible systems as those concerned with certain of the dyes or quinones (16). With quinonoid substances mercaptans may, of course, form addition compounds (see 33) and this is suggestive for observations that the depressant action of p-benzoquinone upon papain is partially irreversible and upon crystalline urease (in solution) completely so (44) (92). Addition reactions may have complicated attempts to evaluate the potentials of mercaptan-dithio systems by the use of the indicator method (32). Worse, these systems have usually defied evaluation of their free energies by the potentiometric method, partly because of technical difficulties with the electrodes. These difficulties seem to be related to the characteristic "affinity" of the reductants for heavy metal derivatives.[6] The obstacles to success (not necessarily insurmountable) are reflected in recent articles upon the subject. (For references, see (11, 32).)

The apparent sluggishness of mercaptan-dithio systems interposes no compelling objection to the conception that their oxidation-reduction state (and a correlated regulation of the activity of certain enzymes) may be under the control of the oxidation-reduction intensity level *in vivo*. It is readily conceivable that cellular dehydrases may aid in bringing such systems into the continuum (15). The reactivation of papain[7] by succinate and dehydrase is suggestive. A by-product of the researches reviewed in this paper is an almost conclusive demonstration of the non-specificity of glutathione in the activation of specified hydrolytic enzymes *in vitro*. A discussion of the specific rôle of this important system in the living cell, although highly tempting, is outside the scope of this review.

It becomes of interest to examine further the validity of some of the more specific methods employed in the enzyme researches. Thus, may a mercaptan, R-S-H, (e.g., cysteine) be regarded as a potential reducing agent for a related dithio compound, R′-S-S-R′ (e.g., a protein

[6] It may be that the oxidant in the *first* step of oxidation of R—S—H to R—S—S—R is a reactive free radicle, R—S— which undergoes a rapid (and under the conditions irreversible) dimerization to R—S—S—R.

[7] Reiss (96) has attempted to measure oxidation-reduction potentials of the "papain system."

with dithio groups)? This may be answered in the affirmative (cf. 8) with the qualification that the reaction should be regarded as essentially reversible; thus

$$R'\text{-}S\text{-}S\text{-}R' + 2RSH \rightleftarrows 2R'SH + R\text{-}S\text{-}S\text{-}R$$

Of obvious importance are the relative concentrations of the components and the conditions of reaction. Du Vigneaud's application of this reaction to the irreversible inactivation of insulin has been cited. If the subsequent oxidation of protein sulfhydryl groups produced in the process reconstitute dithio linkages, the latter might be elaborated in a pattern unlike that in the original "active" protein molecules. (Cf. 132.) A similar effect with urease, papain, cerebrosidase, etc., manifestly would be of less moment since these are active in the reduced state.

Other devices for effecting the reduction of recognized dithio compounds involve the use of sulfides and H_2S, cyanides and HCN (73), sulfite-ion, thiosulfate-ion, suitable treatment with metals, catalyzed hydrogenation, etc. Some of these need further careful study. A critical investigation and evaluation of the sulfite reaction is found in the work of Clarke (17). Most of these reagents have been applied to the enzyme studies.

Interesting variations in the use of reducing agents have been applied by different workers. Maschmann and Helmert (71) (compare (44)) found that papain is activated by l-ascorbic acid, provided that ferrous iron is also present in the reaction mixtures. Otherwise the effect is depressant. Such results are not inconsistent with the versatile reactivity of the vitamine (compare Hanes (41)). Liver cathepsin is also activated (26). That ascorbic acid may function as the reductant of a strictly reversible oxidation-reduction system (11) (4) now seems clear; however, its special characteristics as a reducing agent are conditioned to some extent by other substances also present in reaction mixtures. Purr (90) believes that the activation of papain by l-ascorbic acid plus ferrous ion involves the preliminary reduction of contaminating dithio compounds, which, in turn, reduce and activate the enzyme.

Primarily because of apparent discrepancies encountered in their studies of papain when the substrates and buffer salts are varied, Maschmann and Helmert (72) disagree with a hypothesis which holds that catalytic activity may be regulated through an enzyme's sulfhydryl groups. Difficulties of this character will not be finally resolved until all of the enzymes studied (and even the substrates) are procur-

able in highly purified condition substantially free of the accompanying substances present in crude preparations.[8] Any possible rôle of the latter materials (which would not necessarily coincide with their rôle or that of their precursors *in vivo*) could be ascertained preferably after their identification. The possibility of interaction of reducing agents, etc., with extraneous substances has been indicated (compare 93). The presence of impurities in buffers (e.g., glyoxylic acid as a contaminant in acetate), or an altered reactivity of enzyme-protein molecules as a whole when buffers are varied, may have relevance (compare also 80).

Discrepancies may be more apparent than real. For example, the failure of ferricyanide-ion or phenol-indophenol to effect inactivation of urease (44) (28) or of indophenols to inactivate papain is hardly attributable to an insufficiently "positive" oxidation-reduction level of the systems related to these oxidants. The responses of such enzymes toward reagents are conspicuously dependent upon reaction rates and the conditions which modify rates. For example, at lower temperatures, ferricyanides inactivate hemolysin negligibly slowly; at 37° inactivation is more rapid and complete, but is nevertheless reversed by reduction. A most instructive example is found in the work of Smythe (109). Iodoacetic acid has been considered a valuable and almost specific reagent for its irreversible action upon sulfhydryl groups of certain biologically interesting compounds. From the work of Dickens (20), Michaelis and Schubert (78), and Rapkine (95) it is seen that its action with mercaptans is one of simple alkylation ($RSH + ICH_2COOH \xrightarrow{-HI} R\text{-}S\text{-}CH_2COOH$). Under certain conditions this alkylation of thiol groups is more rapid than of amino groups which, however, are also more or less readily subject to attack. Hellerman, Perkins and Clark observed (43) that iodoacetate ion did not, under the conditions used, cause inactivation of crystalline urease but, mindful of the rate factor, did not feel that this necessarily contradicted their other evidence. Smythe now reports great variations in the rate of interaction of iodoacetate and even simple alkyl mercaptans. The rate of combination of certain proteins is correlated with the disappearance of -SH groups; crystalline urease happens to react *slowly* with iodoacetate and more

[8] In a paper presented before the American Chemical Society at its September, 1936, meeting, A. Walti described the crystallization of ficin, a proteolytic enzyme, possessing anthelminthic properties; he identified the crystalline enzyme as a proteinase of the papain type which, in the absence of supplementary specific "activators," displayed activation characteristics well established for papains.

rapidly with the related compound, iodoacetamide. Other interesting observations include the finding that the activity of urease is abolished after only a fraction of the protein-enzyme's -SH groups have been combined.

It will be recalled that crystalline urease as a whole can combine with ten times the minimum quantity of silver-ion required for its inactivation (111). The reagents employed in this field usually possess a more or less versatile chemistry and rarely are strictly specific in their actions upon any one grouping in a protein molecule.

Bergmann and Ross caution (6), "there are groups, other than those of the -SH $\rightleftarrows$ -S-S- system, having a decisive influence on the activity of the papain constituents." The reviewer is not unmindful of the fascinating complexities of enzyme action. Interpretations are inevitably vulnerable to the advances of controlled experimentation. Workers now seem convinced of the advantages of proceeding in this field on the basis of rational chemical concepts; also, that this approach, where applicable, is much to be preferred to a mere assembly of empirical observations. (Such an assembly may, of course, have served the indispensable function of furnishing suggestions.) An incomplete interpretation can be amplified; an erroneous one discarded.

ARGINASE. Recent studies of the activation of arginase disclose the existence of another important mechanism underlying the regulation of the kinetics of hydrolytic enzymic processes. This concerns the probable rôle of dissociable metal-coördinated complexes (45). A useful historical and descriptive survey of arginase has recently been contributed by Baldwin (3). In an important paper, Krebs (61) has furnished a new conception of the function of d-arginine and of arginase in the conversion of ammonia to urea in the animal organism. Krebs' postulated synthesis of arginine from ornithine and the ammonia and carbonate systems has not been demonstrated in the absence of surviving organized tissue. The transformations are doubtless "coupled" with other processes. So-called desmo-enzymes (see (123) (1)) incapable of transfer to lyo-forms may in part be concerned. The enzyme which accelerates hydrolysis of d-arginine to ornithine and urea is easily extractable in a more or less impure state. Efforts to purify this highly specific enzyme, arginase, have not met conspicuous success.

Earlier studies of the chemical activation of arginase seemed to indicate that organic thiol compounds were activators (99); glutathione was considered to be an essential, specific coferment (125). This idea was abandoned (21) when the effects were found to be variable and it

was determined, in addition, that ferrous ion is a more characteristic activator. The action of the metal ion was especially dependable when there was used, in addition, an organic thiol compound or one of a number of substances such as alloxan (91). It has been held that activators are reducing substances which protect the enzyme against inhibition by oxygen (for references, see 3, 45). Attempts to elucidate the observations of activation in terms of a reversible oxidation-reduction process (123) have met little success.

The responses of arginase to the action of various reagents are strongly dependent upon the nature of extraneous and other materials present. Klein and Ziese state, for instance, (58) (cf. 23) that crude preparations are activated by thiol compounds and other reducing agents and inhibited by oxygen, while "purified" material is not sensitive to oxygen and is inhibited by cysteine, etc. In such circumstances it is not surprising that the results of different workers, with an enzyme possessing marked instability under most conditions used, present many contradictions. These considerations taken with the results of some recent studies (45) (47) upon the nature of the arginine-arginase reaction cast doubt upon the advisability of assuming too generally "that preparations in the form of a *Brei* probably give us a fairly reliable picture of the actual arginase content of any particular tissue" (3).

With a relatively potent liver-arginase, of a high degree of specificity, Hellerman and Perkins (45) found that arginase action neither was significantly altered by mercaptide-forming organomercurials (e.g., (C_6H_5HgOH) nor was enhanced by bisulfites or by such potent reducing agents as organic thiol compounds (e.g., cysteine or reduced glutathione), cyanides, or hydrogen sulfide. These reagents are effective papain activators. Actually the arginase catalysis (at pH 7.4) was inhibited by hydrogen cyanide and drastically suppressed by hydrogen sulfide. Similar inhibitions had been observed by Edlbacher. The activity was readily abolished by the action of hydrogen ion (see (51)) ferric or chromic (46) ion, or by quinone, iodine, etc.

Activation by metal ions. The activating effect of ferrous ion has been emphasized by several authors. The action of the metal ion is assisted by various organic substances, the rôle of which was interpreted (91) in terms of a correlation of apparent oxidation-reduction properties of iron complexes of these materials and the modification of enzyme activity. However, potent reducing agents fail to activate arginase, and it seemed to Hellerman and Perkins (45) that the effect might be related to some other attribute of the ferrous ion, specifically the prop-

erty of coördination (128) (64) (108) with suitable molecules, possibly the enzyme and substrate molecules, themselves. This conception involving no unique property of ferrous ion prompted experimentation with a variety of ions; this disclosed that the reduced ions of certain other transition elements also effected pronounced activation. Such ions are cobaltous, nickelous, and manganous (compare (59) (24)). Of unusual interest are the subsequent observations that *these metal ions accomplish restoration of activity after the enzyme has been treated with inhibiting oxidants;* also that cobaltous ion effects the complete restoration of arginase activity after the enzymic activity has been suppressed by hydrogen sulfide and the excess of the latter removed.

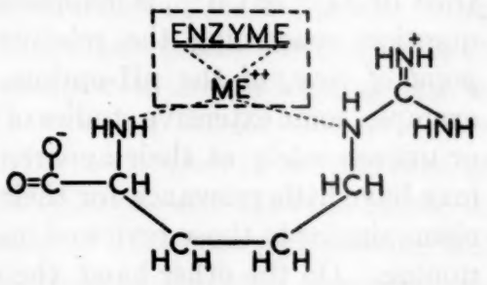
Reference has been made to certain enzymes concerned with cellular oxidation-reduction processes (e.g., oxidases) which contain metallic components. Such catalysts often suffer modification of activity by a more or less reversible combination with cyanide ion, pyridine, nicotine, carbon monoxide, and other substances, all of which are able to form coördinate linkings with metal ions. Differences in the degree of action of these reagents are marked.

The characteristics of activation and inactivation of arginase, a hydrolytic enzyme, suggest a very rough analogy to the chemistry of the hemochromogen types. Thus, arginase may partake of the nature of a heavy metal complex.[9] If the metallic component of such a complex molecule were present in a reduced state when the enzyme is active, inactivation by oxidation might involve simple oxidation of the complex, reversed by suitable reduction, *or* an actual separation of the metallic ion or component from the rest of the molecule, probably coincident with the oxidation of the metal. Hydrogen sulfide or cyanide would depress activity by the formation of an addition compound with the metallo-enzyme, or by disruption of its molecule. An interpretation of activation or reactivation by Co^{++}, etc., would rest upon the assumption that such ions may replace, more or less readily, the metallic atom or component, characteristic of arginase, when that component has been lost to the enzyme by some process which has not injured the rest of the molecule.

Metal coördination in enzymic catalysis. The assumed metal coordination in the activation picture was extended to include the substrate. Such a conception, if acceptable on the basis of available facts, would decidedly enrich our notions concerning the construction, in

[9] The heavy metal content of liver includes iron, copper, manganese, and zinc.

appropriate cases, of labile and more or less dissociable enzyme-substrate intermediates and have particular interest for the problem of enzyme specificity. It would seem also to have some applicability to non-enzymic catalysis, but in neither case should be taken to exclude pertinent physical factors. For clarity, a tentative, schematic representation of the arginine-arginase complex may be given. This depicts the coördinative union of metallo-enzyme (arginase) to d-arginine through the α-amino group, which is probably the substrate's chief "anchoring" group. For simplicity, an electronic representation is omitted. The substituted guanidinium ion has been represented as undergoing resonance (55); such an effect should be modified by the coördinative linking that supposedly involves one of the nitrogen atoms of that ion and the metal atom. Irrespective of the merits of the resonance assumption, the guanidinium ion is doubtless a more symmetrical entity than the classical formula would suggest. In any case the "fixing" of bonds by metal coördination would be associated with promotion of the acceleration of hydrolysis in the direction of formation of ornithine and urea, with concomitant regeneration of the arginase. This involves, also, the interesting question of relative bond strengths, which cannot be treated here.[10]



It is pertinent to inquire whether the shifting equilibria concerned with the interplay of metallo-complexes and whether, in addition, a reversible oxidation-reduction of the metallo-enzyme may be applied to the control of arginase activity *in the cell.* There is no direct evidence, at present. The foregoing discussion raises many interesting questions. Arginase has not yet been isolated and crystallized. The nature of the enzyme's postulated metallic component is unknown. The heavy metal ions that activate or reactive arginase *in vitro* have been found to alter the pH-activity curve of the enzyme characteristi-

[10] In contrast to non-enzymic (hydroxyl-ion catalyzed) hydrolysis of guanidine and its derivatives the enzymatic hydrolysis probably involves the zwitter-ion over most of the enzyme's effective pH range. Arginase does not "attack" esters of d-arginine (cf. (13)); it acts slowly with l-arginine even when large amounts of the enzyme are employed (24). The weight of evidence opposes the assumption that the α-amino group is non-essential; argininic acid and δ-guanidinovaleric acid are not significantly attacked in the presence of a somewhat purified arginase under conditions such that d-arginine is extensively hydrolyzed (47).

cally (47). This provides strong additional evidence in support of the idea that metal coördination is concerned not merely with activation but actually with the functioning of the enzyme.[11] It was observed that the pH optimum for an arginase preparation having maximal activity at pH 7.7 to 9.0 was shifted in the presence of nickelous, cobaltous, and manganous ions, respectively, to pH 6.7 to 7.7, 7.5 to 7.7, and 10. These results are to be considered with reference to the conditions used. Interestingly enough, the variations seem to be related to the corresponding stabilities (gaged by the magnitudes of dissociation constants) of coördination complexes of the effective ions with certain compounds (e.g., arginine; glycine); these were ascertained by means of appropriate potentiometric measurements. Qualitatively, the ions, in the order of stability of their complexes are: $Ni^{++} > Co^{++} > Mn^{++}$. Mn^{++} coördinates only slightly in the more acid range (cf. (69)), and its activating ability in this range (pH 5 to 6.5), in contrast to Ni^{++} or Co^{++}, is inappreciable. Such facts raise an interesting question concerning the relative importance, *from the physiological point of view*, of the pH-optima, as such, of certain enzymes. For example, some extensive studies of the behavior of enzymes like arginase or uricase solely at their apparent optima in the more alkaline range may have little relevance for their characteristics *in vivo* where mechanisms similar to those reviewed may be available for their efficient functioning. On the other hand, the advantages of observation of enzyme activation over a widely varying pH range with due regard for such variables as the ionic strength, nature of buffer salts, etc., are not in question.

"Artificial" arginase. It has also been found (47) that the postulated non-metallic or "donor" component of arginase may be replaced by some constituent of certain preparations of jack bean urease (but not by highly recrystallized urease) in the enzymatic hydrolysis of d-arginine. This entity is not identical with the corresponding constituent (a protein?) of liver-arginase. However, the characteristics of the kinetics of arginine hydrolysis with either enzyme (plus Co^{++}) are similar, e.g., "first-order" at pH 7.5; furthermore, the striking specificity of arginase is retained in the new catalyst.

RELATED PROBLEMS. The apparent rôle of certain coördinating metal ions in the reversible activation of arginase has not only a sig-

[11] In contrast, the evidence at present available concerning the rôle of substituent —SH groups in urease, cathepsins, etc., has dealt more exclusively with the regulatory function.

nificant bearing upon the general position of metals in enzymic catalysis; it possesses peculiar interest for several closely related problems. For the crystallization of insulin, Scott finds zinc ion to be essential (104). Zinc is an integral part of crystalline insulin (105). It may be "replaced" by Co^{++}, Ni^{++}, or Cd^{++}. Of great interest is the question whether zinc, a constituent of the pancreas and liver, may be concerned with regulatory functions.

Sumner's jack bean hemagglutinin (113) is inactivated by acidification, which separates metal ion from the active protein; reactivation is effected by the use of certain metal ions. The latter are apparently integral parts of the molecules of crystalline protein.

The "antagonistic" effect of Ni^{++}, Co^{++} or Mn^{++} against the action of calcium ion in the conversion of prothrombin to thrombin seems to be concerned with equilibrium reactions (48); the order of effectiveness is again $Ni^{++} > Co^{++} > Mn^{++}$; the elaboration of thrombin may well involve a catalytic process in which calcium ion plays the rôle of an orienting or coördinating ion (involving prothrombin and a platelet or tissue catalyst) analogous to that assigned to reduced metal ions in the arginase-arginine picture.

Certain peptidases have been found to be subject to a depression of their activity by cyanides. Polyaminophosphatase has been shown to be strongly inhibited by glutathione, etc. The phosphatases (cf. 22) and nucleotidases of several organs seem to be similarly sensitive. In the absence of abundant experimental data a number of mechanisms are readily invoked. However it may be fair to inquire to what extent the experiences with arginase may have application to some of these cases. Studies of the protective effect (cf. 10) of amino acids in phosphatase action may conceivably involve the removal of inhibiting heavy metal ions. It may be recalled that sensitiveness to certain metal ions is practically a general characteristic of enzymes, although there are marked differences in degree and specificity of action; even arginase is inactivated and partially precipitated by silver or cupric ion or by mercuric chloride.

The "co-enzyme action" of Mg^{++} for the effectiveness in cellular processes of enzymic catalysis of certain phosphate-ester syntheses and hydrolyses is well recognized. In a similar sense, Ca^{++} might be termed a co-enzyme in the conversion of prothrombin to thrombin, and the supposed metallic component of arginase, a co-enzyme for d-arginine hydrolysis; this assigns, however tentatively, a rational function for such ionic coenzymes (cf. 1), the identity of which as

integral constituents of the enzymes or enzyme-substrate compounds is sometimes confused by the phenomenon of dissociation.[12]

CONCLUSION

Little has been said in this review concerning the specific rôles of individual activators and inactivators. On the contrary, there has been pointed out the versatile chemistry of many of the reagents employed. For example, reduced glutathione, widely distributed in plant and animal tissues, may be considered to be the reductant of a relatively negative oxidation-reduction system; its reducing properties have been related as such to the reversible activation of certain enzymes; the substance is competent, also, to effect the destructive reduction of certain principles (e.g., insulin) by attack upon their dithio linkages and likewise to reduce keratins. It also shares with numerous other compounds the property of combining with appropriate metal ions in the formation of complexes. Thus it may function seemingly to "protect" ferrous ion against oxidation in the Fe^{++} activation of arginase or to reactivate certain enzymes inhibited by heavy metal ions, or to suppress the activity of a number of metal-containing enzymes. In general, the complexes possess also oxidation-reduction properties (76) (117) characteristic of themselves. Similar considerations apply to other thiol-dithio systems. Hydrogen cyanide is a reducing agent and also suppresses certain inhibiting heavy metal ions by complex-formation; the latter property may also permit its reversible combination with certain metallo-enzymes (e.g., catalase; peroxidase). Because of this complexity and for other reasons it has been deemed advisable to develop the themes of reversible inactivation in terms of abundant, cumulative evidence, and to omit detailed discussion of cases in which this position has not yet been attained.

There have been discussed the salient data bearing 1, upon the reversible inactivation by oxidation-reduction and by mercaptide-forming reagents of specified hydrolytic enzymes, presumably through reversible chemical actions upon substituent thiol groups of these enzymes; 2, upon the rôle of specific metal-coördinative processes in the reversible inactivation of arginase; 3, upon the rôle of reversible oxidation-reduction and dissociable metal complexes in the control of the kinetics of certain biological hydrolytic processes. The observations and interpretations have been considered with respect to several related problems of general physiological interest.

[12] Compare the modification of salivary amylase action by halide ions, especially chloride (see 40).

REFERENCES

(1) ALBERS, H. Angew. Chem. **49**: 448, 1936.
(2) AMBROS, O. AND A. HARTENECK. Ztschr. physiol. Chem. **181**: 24, 1929; **184**: 93, 1929.
(3) BALDWIN, E. Biol. Rev. **11**: 247, 1936.
(4) BALL, E. G. J. Biol. Chem. **118**: 219, 1937.
(5) BERGMANN, M. AND COLLABORATORS. Science **79**: 439, 1934; J. Biol. Chem. **109**: 325, 1935; **111**: 659, 1935; **114**: 711, 1936; Science **84**: 89, 1936.
(6) BERGMANN, M. AND W. F. ROSS. J. Biol. Chem. **114**: 722, 1936.
(7) BERSIN, T. Ztschr. physiol. Chem. **222**: 177, 1933.
(8) BERSIN, T. Ergebn. Enzymforschung. **4**: 68, 1935.
(9) BERSIN, T. AND W. LOGEMANN. Ztschr. physiol. Chem. **220**: 209, 1933.
(10) BODANSKY, O. J. Biol. Chem. **114**: 273; **115**: 101, 1936.
(11) BORSOOK, H. AND CO-WORKERS. J. Biol. Chem. **117**: 237, 281, 1937.
(12) BRØNSTED, J. N. Chem. Rev. **5**: 231, 1928.
(13) CALVARY, H. O. AND W. D. BLOCK. J. Biol. Chem. **107**: 155, 1934.
(14) CLARK, W. M. The determination of hydrogen ions. 3rd ed. Baltimore, 1928.
(15) CLARK, W. M. Medicine **13**: 207, 1934. (Harvey Lecture, 1933.)
(16) CLARK, W. M. AND CO-WORKERS. Public Health Reports, Bull. **151**: 1928.
(17) CLARKE, H. T. J. Biol. Chem. **97**: 235, 1932.
(18) COHEN, B. Cold Spring Harbor Symposia on Quant. Biol. **1**: 2, 1933.
(19) COHEN, B. AND H. SHWACHMAN. J. Bact. **31**: 67, 1936; Proc. Soc. Exper. Biol. and Med. **35**: 586, 1937.
(20) DICKENS, F. Biochem. J. **27**: 1141, 1933.
(21) EDLBACHER, S., J. KRAUS AND G. WALTER. Ztschr. physiol. Chem. **206**: 65, 1932.
(22) EDLBACHER, S. AND KUTSCHER. Ztschr. physiol. Chem. **207**: 1, 1932.
(23) EDLBACHER, S., J. KRAUS AND F. LENTHARDT. Ztschr. physiol. Chem. **217**: 89, 1933.
(24) EDLBACHER, S. AND A. ZELLER. Ztschr. physiol. Chem. **242**: 253, 1936.
(25) EULER, H. VON AND O. SVANBERG. Fermentforschung **3**: 330, 1920.
(26) EULER, H. VON, P. KARRER AND F. ZEHENDER. Helv. Chim. Acta **17**: 157, 1934.
(27) EULER, H. VON, H. ALBERS AND F. SCHLENK. Ztschr. physiol. Chem. **234**: I; **237**: I, 1935.
(28) FISCHGOLD, H. Biochem. J. **38**: 406, 1934.
(29) FLEMING, A. Proc. Roy. Soc. London, Series **B 93**: 306, 1922.
(30) FRANKEL. J. Biol. Chem. **31**: 201, 1917.
(31) FREUDENBERG, K. AND H. EYER. Ztschr. physiol. Chem. **213**: 226, 1932.
(32) FRUTON, J. S. AND H. T. CLARKE. J. Biol. Chem. **106**: 667, 1934.
(33) GIBSON, D. T. Chem. Rev. **14**: 431, 1934.
(34) GLASSTONE, S. Recent advances in physical chemistry. 2nd ed. Philadelphia, 1933.
(35) GODDARD, D. R. AND L. MICHAELIS. J. Biol. Chem. **106**: 605, 1934; **112**: 361, 1935.
(36) GRASSMANN, W. In C. Oppenheimer, Die Fermente und ihre Wirkungen, Leipsic, 3rd ed. **3**: 1012–1021, 1929; Ergebn. Enzymforsch. **1**: 152, 1932.

(37) GRASSMANN, W., H. DYCKERHOFF AND O. VON SCHOENEBECK. Ztschr. physiol. Chem. **186**: 183, 1929–30; **194**: 124, 1930.
(38) GRASSMANN, W., O. VON SCHOENEBECK AND H. EIBELER. Ztschr. physiol. Chem. **194**: 260, 1930.
(39) GULLAND, J. M. AND S. S. RANDALL. Biochem. J. **29**: 378, 391, 1935.
(40) HALDANE, J. B. S. Enzymes. Monographs on Biochemistry. London and New York, 1930.
(41) HANES, C. S. Biochem. J. **29**: 2588, 1935.
(42) HELLERMAN, L. AND M. D. NEWMAN. J. Am. Chem. Soc. **54**: 2859, 1932.
(43) HELLERMAN, L., M. E. PERKINS AND W. M. CLARK. Proc. Nat. Acad. Sci. **19**: 855, 1933.
(44) HELLERMAN, L. AND M. E. PERKINS. J. Biol. Chem. **107**: 241, 1934.
(45) HELLERMAN, L. AND M. E. PERKINS. J. Biol. Chem. **112**: 175, 1935.
(46) HELLERMAN, L. AND M. E. PERKINS. Unpublished work, 1935.
(47) HELLERMAN, L. AND C. C. STOCK. J. Am. Chem. Soc. **58**: 2654, 1936.
(48) HELLERMAN, L., H. MILSTONE AND W. H. CARNES. To be published.
(49) HOOGERHEIDE, J. C. Doctoral Dissertation. Delft, 1935.
(50) HOPKINS, F. G. J. Biol. Chem. **84**: 269, 1929.
(51) HUNTER, A. AND J. A. DAUPHINEE. Quart. J. Exper. Physiol. **23**: 119, 1933.
(52) JACOBY, M. Fermentforsch. **10**: 1, 1928; Biochem. Ztschr. **259**: 211, 1933.
(53) JØRGENSEN, H. Biochem. Ztschr. **280**: 1, 1935.
(54) JOWETT, M. AND J. H. QUASTEL. Biochem. J. **27**: 486, 1933.
(55) JUKES, T. H. AND G. E. K. BRANCH. Science **80**: 228, 1934.
(56) KEILIN, D. Ergebn. Enzymforsch. **2**: 239, 1933.
(57) KITAGAWA. J. Biochem. (Japan) **10**: 197, 1929.
(58) KLEIN, G. AND W. ZIESE. Ztschr. physiol. Chem. **211**: 23, 1932; **222**: 187, 1933; **229**: 209, 1934.
(59) KLEIN, G. AND W. ZIESE. Klin. Wchnschr. **14**: 205, 1935.
(60) KREBS, H. A. Biochem. Ztschr. **220**: 289, 1930; **238**: 174, 1931.
(61) KREBS, H. A. AND K. HENSELEIT. Ztschr. physiol. Chem. **210**: 33, 1932.
(62) KUHN, R. Nature **135**: 185, 1935.
(63) KUNITZ, M. AND J. H. NORTHROP. J. Gen. Physiol. **19**: 991, 1936.
(64) LEWIS, G. N. Valence and the structure of molecules. New York, 1923.
(65) LEWIS, G. N. AND M. RANDALL. J. Am. Chem. Soc. **43**: 1112, 1921. Thermodynamics. New York, 1923.
(66) LINDERSTRØM-LANG, K. AND F. DUSPIVA. Ztschr. physiol. Chem. **237**: 131, 1935. (66a) Linderstrøm-Lang, K. Ann. Rev. Biochem. **6**: 43, 1937.
(67) LIPMANN, F. Biochem. Ztschr. **265**: 133, 1933.
(68) LOHMANN, K. Biochem. Ztschr. **254**: 332, 1932.
(69) MAIN, R. AND C. L. A. SCHMIDT. J. Gen. Physiol. **19**: 127, 1935.
(70) MASCHMANN, E. AND E. HELMERT. Ztschr. physiol. Chem. **219**: 100, 1933.
(71) MASCHMANN, E. AND E. HELMERT. Ztschr. physiol. Chem. **224**: 56, 1934.
(72) MASCHMANN, E. AND E. HELMERT. Ztschr. physiol. Chem. **231**: 51, 1935; Biochem. Ztschr. **277**: 97; **279**: 213, 1935.
(73) MAUTHNER, J. Ztschr. physiol. Chem. **78**: 28, 1912; Ztschr. f. Biol. **42**: 184, 1901.
(74) MENDEL, L. B. AND A. F. BLOOD. J. Biol. Chem. **8**: 177, 1910.

(75) MEYER, K., R. THOMPSON, J. W. PALMER AND D. KHORAZO. J. Biol. Chem. **113:** 303, 1936.
(76) MICHAELIS, L. Oxydations-Reductions potentiale. 2nd ed. Berlin, 1933.
(77) MICHAELIS, L. AND H. DAVIDSOHN. Biochem. Ztschr. **36:** 280, 1911.
(78) MICHAELIS, L. AND M. P. SCHUBERT. J. Biol. Chem. **106:** 331, 1934.
(79) MICHAELIS, L. AND K. G. STERN. Biochem. Ztschr. **240:** 192, 1930.
(80) MIRSKY, A. E. AND M. L. ANSON. J. Gen. Physiol. **19:** 427, 439, 1936.
MIRSKY, A. E. J. Gen. Physiol. **19:** 559, 1936.
(81) MOTHES, K. Naturwissenschaften **20:** 102, 1932; **21:** 883, 1933.
(82) MYRBÄCK, K. Ztschr. physiol. Chem. **158:** 160, 1926.
(83) NEILL, J. M. J. Exper. Med. **44:** 199, 1926.
(84) NORD, F. F. AND R. WEIDENHAGEN. Ergebn. d. Enzymforschung **1–5:** 1932–36.
(85) NORTHROP, J. H. Biol. Rev. **10:** 263, 1935; The Harvey Lectures **30:** 229, 1934–35.
(86) OPPENHEIMER, C. Die Fermente und ihre Wirkungen. Leipsic, 5th ed. **2:** 782, 1926.
(87) PERLZWEIG, W. A. Science **76:** 435, 1932; Proc. Am. Soc. Biol. Chemists 77, 1933.
(88) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Methods: 545, Baltimore, 1932.
(89) PIRIE, N. W. Biochem. J. **25:** 614, 1931.
(90) PURR, A. Biochem. J. **29:** 5, 13, 1935.
(91) PURR, A. AND L. WEIL. Biochem. J. **28:** 740, 1934.
(92) QUASTEL, J. H. Biochem. J. **27:** 1116, 1933.
(93) QUASTEL, J. H. Ann. Rev. Biochem. **5:** 25, 1936.
(94) QUASTEL, J. H. AND A. H. WHEATLEY. Biochem. J. **26:** 2169, 1932.
(95) RAPKINE, L. Compt. rend. soc. biol. **112:** 790, 1933.
(96) REISS, P. Compt. rend. soc. biol. **120:** 908, 1935.
(97) RUCHELMANN. Biochem. Ztschr. **259:** 358, 1933.
(98) SACHS, G. Ann. Chem. **433:** 154, 1923.
(99) SALASKIN, S. AND L. SOLOWJEW. Ztschr. physiol. Chem. **200:** 529, 1931; Biochem. Ztschr. **250:** 503, 1932.
(100) SCHMIDT, E. G. J. Biol. Chem. **78:** 53, 1928.
(101) SCHOCK, E. D., H. JENSEN AND L. HELLERMAN. J. Biol. Chem. **111:** 553, 1935.
(102) SCHÖNBEIN. J. f. prak. Chem. **105:** 202, 1868.
(103) SCHUBERT, M. P. J. Biol. Chem. **111:** 671, 1935.
(104) SCOTT, D. A. Biochem. J. **28:** 1592, 1934.
(105) SCOTT, D. A. AND A. M. FISHER. Biochem. J. **29:** 1048, 1935.
(106) SEALOCK, R. R. AND V. DU VIGNEAUD. J. Pharmacol. Exper. Therap. **54:** 433, 1935.
(107) SHWACHMAN, H., L. HELLERMAN AND B. COHEN. J. Biol. Chem. **107:** 257, 1934.
(108) SIDGWICK, N. V. Electronic theory of valency. London, 1927; Chemistry and Industry **46:** 799, 1935.
(109) SMYTHE, C. V. J. Biol. Chem. **114:** 601, 1936.

(110) Sørensen, S. P. L. Compt. rend. Lab. Carlsberg **8:** 1, 1909; **8:** 396, 1909; Biochem. Ztschr. **21:** 131, 201, 1909; **22:** 352, 1909.
(111) Sumner, J. B. Ergebn. d. Enzymforschung **1:** 295, 1932. Leipsic. J. Nutrition, **6:** 103, 1933.
(112) Sumner, J. B. and L. O. Poland. Proc. Soc. Exper. Biol. and Med. **30:** 553, 1933.
(113) Sumner, J. B. and S. F. Howell. J. Biol. Chem. **115:** 583, 1936.
(114) Swensen, T. L. J. Assoc. Official Agr. Chem. **19:** 373, 1936.
(115) Thannhauser, S. J. and M. Reichel. J. Biol. Chem. **113:** 311, 1936.
(116) Theorell, H. Biochem. Ztschr. **272:** 155, 1934.
(117) Thimann, K. V. Rev. gen. Sci. **45:** 593, 1934; **46:** 71, 1935.
(118) Traub, H. P., T. R. Robinson and H. E. Stevens. Science **83:** 569, 1935.
(119) du Vigneaud, V., A. Fitch, E. Pekarek and W. W. Lockwood. J. Biol. Chem. **94:** 233, 1931–32.
(120) Vines. Ann. Botany, **17:** 606, 1903; **19:** 149, 1905.
(121) Voegtlin, C. and M. E. Maver. Public Health Reports **47:** 711, 1932.
(122) Waldschmidt-Leitz, E. Naturwissenschaften **17:** 85, 1929; Ztschr. physiol. Chem. **188:** 17, 1930.
(123) Waldschmidt-Leitz, E. J. Soc. Chem. Ind. **55:** 620, 1936.
(124) Waldschmidt-Leitz, E., A. Purr and A. K. Balls. Naturwissenschaften **18:** 644, 1930; Ztschr. physiol. Chem. **198:** 260, 1931.
(125) Waldschmidt-Leitz, E. and co-workers. Naturwissenschaften **19:** 964, 1931; **21:** 848, 1933; Ztschr. physiol. Chem. **214:** 75, 1933; **215:** 64, 1933.
(126) Warburg, O. and W. Christian. Biochem. Ztschr. **254:** 438, 1932; **258:** 496, 1933.
(127) Warburg, O., W. Christian and A. Griese. Biochem. Ztschr. **282:** 157, 1935; **285:** 156, 1936.
(128) Werner, A. Neure Anschauungen auf dem Gebiete der anorganischen Chemie. Brunswick, 2nd ed., 1909.
(129) Whitmore, F. C. Organic compounds of mercury. New York, 1921.
(130) Willstätter, R. Untersuchungen über Enzyme. 2 vol., Berlin, 1928.
(131) Willstätter, R., W. Grassmann and O. Ambros. Ztschr. physiol. Chem. **151:** 286, 307, 1925.
(132) Wintersteiner, O. J. Biol. Chem. **102:** 473, 1933.
(133) Woodward, G. E. J. Biol. Chem. **109:** 1, 1935.
(134) Wurmser, R. Ann. Rev. Biochem. **1:** 55, 1932.
(135) Zeile, K. Ztschr. physiol. Chem. **236:** 212, 1935.